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EVALUATION OF TECHNIQUES FOR ENVIRONMENTAL MONITORING OF SALMON FARMS IN TASMANIA

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Summary

This study assessed several environmental variables/techniques for their suitability as indicators of organic enrichment from salmon farms and for inclusion in an industry-wide monitoring program, i.e. are practicable, inexpensive and scientifically credible. The general conclusion was that no one variable was sufficiently reliable as an indicator of environmental condition, and that several variables should be routinely monitored. Also, the monitoring program should be regularly assessed and improved as more data become available.

Of the physical/chemical variables investigated, only redox was considered to be suitable. Organic matter, as measured by Loss on Ignition, was found to be highly correlated with sediment particle size but not with the level of organic input, and %C and %N were suitable indicators of organic matter only at very high concentrations. Similarly, stable isotopes of nitrogen and carbon in fish food were effective indicators only at high levels of organic enrichment.

The community structure of the macrobenthic invertebrate fauna was found to be a sensitive and reliable measure of sediment condition. Multivariate analysis of the data was able to separate the fauna into major, moderate and minimal impact levels. In degraded conditions, the ubiquitous polychaete, *Capitella capitata* sp. complex, occurred at very high densities and may be suitable as an indicator species. Identification of organisms to family level was found to be sufficient to show levels of organic enrichment; however identification to species level provided more subtle information on the condition of the sediment. The number of benthic infaunal samples required to reliably assess an impact was suggested to include monitoring at fixed sites, at sites that have been determined to have had relatively high levels of impact and at several reference sites.

Video recordings were found to be suitable for a monitoring program because they provide a relatively inexpensive, instant, permanent record of sediment conditions that is readily interpreted by stakeholders. Degraded conditions were clearly evident in the video footage, in particular from the presence of *Beggiatoa* bacterial mats, black sediments, waste food and faeces, and from the decline in macroalgal cover at specific locations. Video recordings identified severe impacts similar to the macrofauna, but moderate levels of impact were not so obvious.

1. Introduction

In a shallow unstressed marine environment organic matter deposited on the sea bed is broken down by benthic protozoan, macrofaunal, meiofaunal and microbial communities. Fish farming, however, has the potential to upset the natural rates of decomposition because excessive loadings of fish food and faeces on the bottom can result in oxygen depletion and associated detrimental effects on the sediment ecosystem. The effects of organic matter build up around salmon farms have been well documented (e.g. Gowen, 1991; Wu, 1995; Black *et al.*, 1996). When the oxygen supply is limited, sulphate-reducing and methanogenic bacteria dominate, leading to the production of toxic substances. These products, hydrogen sulphide and methane or their derivatives, can decimate the marine fauna and flora and severely affect the health of cultured fish. Such deleterious effects on the sediment ecosystem can be avoided by good farming practises, whereby organic wastes, in particular waste foods, are reduced to a minimum and the cages of fish are removed before the sediment becomes too degraded.

In Tasmania, farming of Atlantic salmon (*Salmo salar* L) has developed quickly since the first trials in 1985. Production in 1996 was estimated at 6,000 metric tons and rose to approximately 10,000 tons by 2000. The Tasmanian state government, the aquaculture industry and communities living in salmon farming areas have all recognised the need to monitor the environment around salmon farms to ensure that the industry develops in an ecologically sustainable manner. Meetings between these stakeholders were held to develop an industry-wide environmental monitoring program for salmon farms. At these meetings there was considerable debate over which and how often environmental parameters should be monitored . Industry was concerned about the cost-effectiveness and usefulness of monitoring several environmental variables, in particular benthic biota and redox. As a consequence of these discussions, it was agreed that research would be conducted to develop an environmental monitoring program for salmon farming in Tasmania.

The objectives of this study were:

- To investigate the suitability of several environmental variables and techniques for monitoring the sediment around salmon farms.

- To assist in the development of a cost-effective, practicable and scientifically credible monitoring program relevant to Tasmanian environmental conditions.

The variables measured as part of this study were based on a review of environmental monitoring programs conducted in other countries. Several of these studies have investigated appropriate measures for evaluating organic enrichment from fish farms, (e.g. Cochrane, 1995; Henderson and Ross, 1995; GESAMP, 1996), and have generally recommended benthic infaunal composition and abundance as one of the most sensitive measure of organic enrichment. However, analysis of invertebrate community composition is time consuming and requires considerable expertise; hence it is expensive to undertake. Video assessment of the environment around marine farming operations has also become commonplace; it has the advantage that is more rapid and inexpensive to conduct and that a permanent visual record is made of the environmental

conditions. Other physical-chemical variables commonly recorded in salmon farm monitoring programs elsewhere include organic content of the sediment and redox.

Environmental variables monitored on salmon farms vary between countries, partly because of differing environmental conditions and partly because of political and historical considerations. It was thus necessary to conduct research in Tasmania to determine the best variables to monitor for Tasmania's environmental and social conditions, and also to be able to recommend levels of acceptable/unacceptable impact on the Tasmanian marine environment. In this report we compare the suitability and effectiveness of video recordings of the seabed with benthic infaunal composition and abundance, and several physical/chemical variables, for an industry-wide salmon farm environmental monitoring program.

This research project has been developed around Management Controls for marine farming which are enforceable under the Tasmanian Marine Farming Planning Act 1995. These controls stipulate that "There must be no unacceptable environmental impact 35m outside the boundary of the marine farming lease area. Relevant environmental parameters must be monitored in the lease area, 35m from the boundary of the marine farm lease area and at any control site(s) in accordance with the requirements specified in the relevant marine farming licence." (Marine Farming Development Plans for Tasmania, available at http://www.dpif.tas.gov.au/domino/DPIF/Fishing.nsf). For this reason we investigated environmental variables at the boundaries of the farm, as well as along transects running out from cages of fish.

However, changes in water column parameters, e.g. nutrients such as nitrates and phosphates, or chlorophyll a concentrations, were not examined because they were being investigated in a separate Huon Estuary Study (CSIRO Huon Estuary Study Team, 2000). Antibiotics and chemicals in sediments as a consequence of salmon farming also were not examined because they were not considered a priority at the time.

2. Sites And Sampling Procedures

2.1 Sites

Environmental monitoring methods were investigated at two Atlantic salmon farm sites in south eastern Tasmania - at Hideaway Bay in the Huon Estuary and Parsons Bay, Nubeena (Fig. 2.1). These sites were chosen to enable testing of the monitoring methods under different environmental conditions. The farm at Hideaway Bay, near the mouth of the Huon River, is located in a protected estuarine environment. The other farm at Nubeena is located in a marine inlet, which is flushed by coastal waters and periodically scoured out by storm surge. The tidal range at both sites is approximately 1 m.

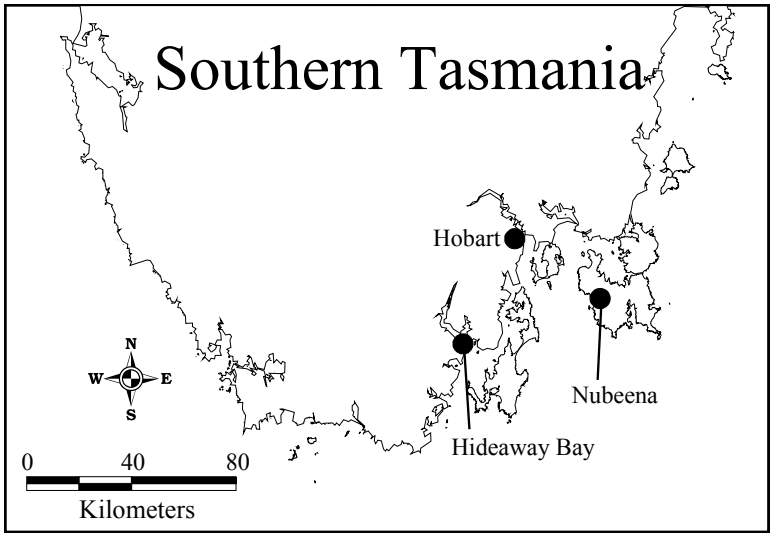


Fig. 2.1. Location of farm sites in south eastern Tasmania

2.1.1 Hideaway Bay

Details of the Hideaway Bay Marine Farm #93 are given in the Huon River and Port Esperance Marine Farming Development Plans (DPIF, 1996a) and in the Environmental Assessment of Marine Farm #93 (Mitchell *et al.*, 1997). In summary, the Hideaway Bay farm is located in water depths ranging from the shore to approximately 34 m, with most of the cages in the deeper areas of the lease at 10 to 34 m. Current measurements taken at 5 m depth sub-surface over three months indicated that the predominant water flow was parallel to the shore, not tidally driven, and the current speed was low, averaging 3.6 $cm.sec^{-1}$ (Mitchell *et al.*, 1997). During this three month period the surface salinity ranged from 27.9 to 34.6 ‰. Annual average monthly water temperatures from 1988 to 1994 ranged from 12 - 19°C (DPIF, 1996a). The substrate is predominantly silty sand in the shallow areas and becomes finer silt/clay at approximately 17 m. (Mitchell *et al.*, 1997).

Salmon farming commenced in 1986 over an area of 8.8 ha, and a further 5 ha was granted in 1993. When this research project commenced in spring 1996 the farm occupied an area of approximately 25 ha, including an area of 12 ha enclosed by netting to prevent the entry of seals. During the sampling period in 1997 the farm area expanded to 40 ha.

2.1.2 Nubeena

Environmental and farm history information for the salmon farm at Nubeena, (Marine Farm #75 and Marine Farming Zone No. 14C) is available in the Tasman Peninsula and Norfolk Bay Marine Farming Development Plan (DPIF, 1996b). This farm is in water depths of 10-20 m, water temperature range of 8-17°C, and salinity of 32-33‰. Current speeds measured at 5 m below the surface varied between 2 and 10 $cm.sec^{-1}$ for 45 % of the time, and 0 $cm.sec^{-1}$ for 51% of the time, with direction of flow predominantly along the shore. This site was first used in 1980 to trial seawater cage culture of rainbow trout, and changed to the commercial culture of Atlantic salmon in 1986. The farm has been expanded several times to an area of 10.9 ha (DPIF, 1996b). In 1997 this farm was slightly relocated and the farm was extended further out into Parsons Bay with an increase in area to12.4 ha.

2.2 Sampling Protocol

An initial objective of this research was to assess the sampling protocol proposed by the Marine Farming Branch of the Department of Primary Industry and Fisheries for an industry-wide monitoring program. Consequently the design of sampling transects and selection of sites has largely followed their recommendations. Their sampling protocol required surveys to be conducted along transects which crossed lease boundaries both parallel to, and across, the direction of prevailing current flow. These boundary transects (B's) were 60 m in length; they started 10 m within the lease boundary and extended perpendicular to the boundary for 50 m. Sample sites were located at 0 m, 45 m and 60 m along the transects, with the 45 m site corresponding to the 35 m monitoring point for compliance to legislation. These sites were chosen to investigate environmental effects near the boundary, at the 35 m compliance point, and at a greater distance away from the lease area. Reference sites were located at least 100 m away from the farms in areas where they were unlikely to be affected by farming activities. All transect lines and sites were located to within 5 m accuracy using real-time differential corrected GPS (DGPS) coordinates at the surface.

2.2.1 Hideaway Bay

At Hideaway Bay six boundary transects (B1-B6), and 4 reference sites (R1-R4) were sampled (Fig. 2.2). A transect inside the farm (F) which extended from the edge of a stocked cage to 60 m away was also sampled from the second sampling event onwards. This site had been periodically stocked with fish since July 1995, and was fallowed for 4 months prior to sampling. The cage was stocked with smolt at a biomass of approximately 10 tonnes just after the commencement of sampling, and was harvested at approximately 40 tonnes. This site was occupied during most of the twelve month sampling period. Locations of other stocked cages are not shown in Fig. 2.2 because

cages of fish were regularly moved around the farm during the sampling period. Sampling started and ended in spring and occurred at 0, 3, 6, 9 and 11 months, but some results at 0 months have not been included because of inconsistencies in sampling protocols between this and later samplings.

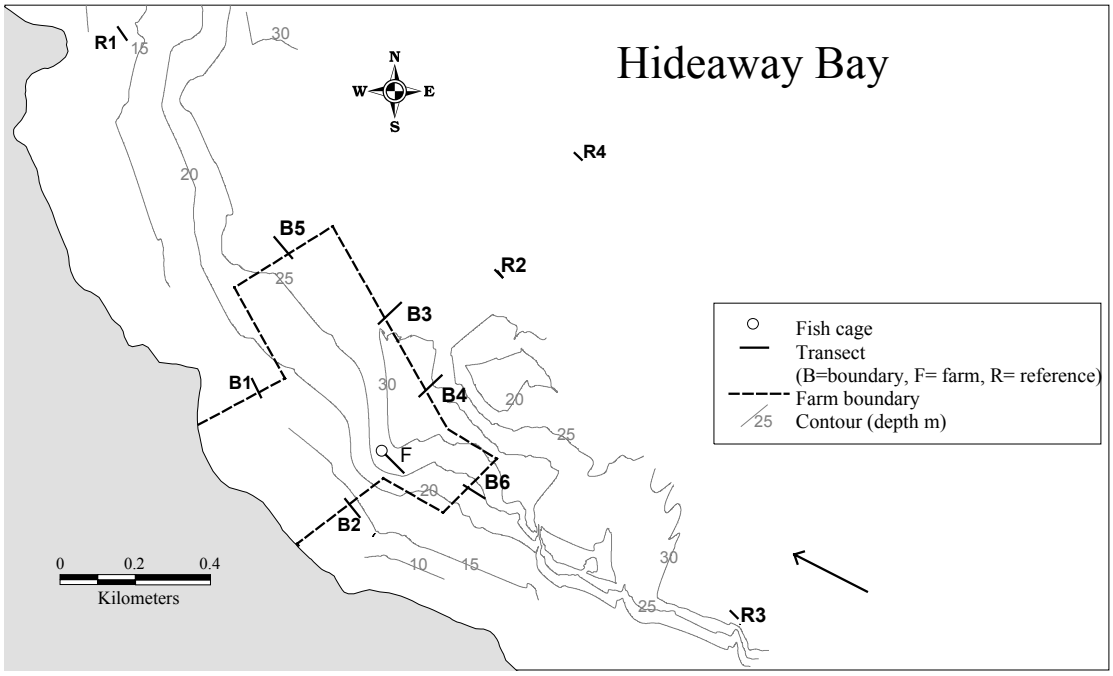


Fig. 2.2. Map of the salmon farm at Hideaway Bay showing locations of transects and reference sites. Arrow indicates predominant direction of current flow.

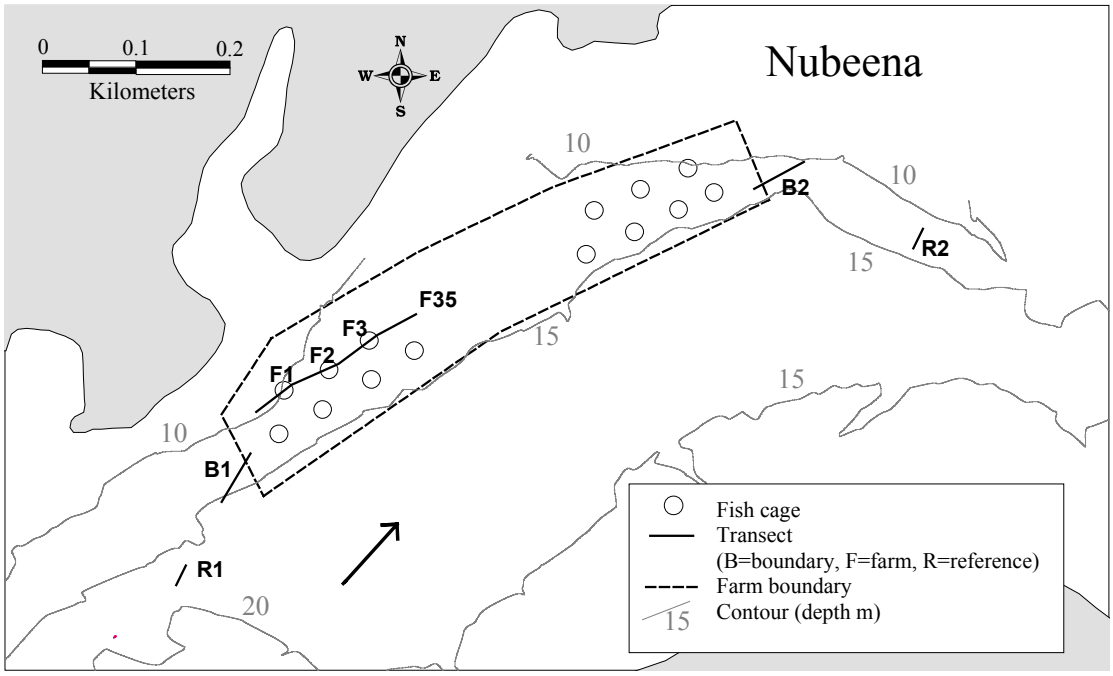


Fig. 2.3. Map of the salmon farm at Nubeena showing locations of transects and reference sites. Arrow indicates predominant direction of current flow.

2.2.2 Nubeena

Boundary transects (B1 and B2) were located at the upstream and downstream boundaries (Fig. 2.3). Additionally, a long farm transect extended around three cages to 35 m east of the third cage. Samples were collected at the edge of cages F1, F2, F3, at 35 m from cage F3 (F35), at sites 0 m, 45 m and 60 m along the boundary transects, and at reference sites R1 and R2 at 0 and 5 months, and at a reduced number of sites at 10 months. Sampling at Nubeena occurred at 0 (spring), 5 (autumn) and 10 (spring) months. Cages were stocked with fish 8 weeks before the commencement of sampling, and were empty (fallowed) for approximately 7 weeks prior to the last sampling. Each of the cages was stocked with approximately 13,000 fish at a mean size of 75 g, and was harvested 10 months later at a mean size of 2.3 kg; salmon biomass increased by 23.3 - 28.5 tons during this time. The location of cages within the farm during the sampling period is shown in Fig. 2.3.

Salmon production in 1996/97 was 880 tonnes at Hideaway Bay and 350 tonnes at Nubeena. Although total production differed between the two farms, production per hectare of lease area was similar (35 and 28 tonnes per ha, respectively) and food conversion ratios were also comparable. The cages selected held salmon at similar stocking densities, with a maximum of 10-12 kg m^{-3}.

2.3 Pilot Study - Comparison Of Sample Techniques

Based on experience, diver sampling was considered to be the most effective technique for collecting sediment samples for benthic infauna, however, it was not possible to collect all samples by diving because of prohibitive depths at Hideaway Bay. In the initial sampling at both Nubeena and Hideaway Bay several sampling techniques were examined to determine whether results collected using different sampling techniques could be compared. These included - diver collected core samples, small Van Veen grab samples, large Van Veen grab samples, core sub-samples from small and large Van Veen grab samples and Ekman grab samples. Two or more techniques were evaluated at each farm, with three replicate samples from each technique collected. Each technique was assessed using several univariate descriptors of the benthic invertebrate community (total number of species, number of individuals per m^2, Shannon diversity index and Inverse Simpson index). The results were compared using analysis of variance (ANOVA).

ANOVA showed no significant difference ($P < 0.05$) between any of the indices when the diver cores, Ekman grab or sub-sample from the small Van-Veen grab were employed. However, the diver cores, small Van-Veen grab, the large Van-Veen grab and sub-samples from this grab, were significantly different for number of species. Additionally, all univariate indices were significantly different between the Ekman grab and large Van-Veen grab.

2.4 Sampling Techniques

After this pilot study, sediment samples for physical/chemical analysis were collected remotely in deep water using a Craib corer (Fig. 2.4) with a perspex core of 50 mm OD and 240 mm height. In shallow water, divers collected cores using the internal cores

from the Craib corer. Benthic infauna was sampled in deep water using a small Van Veen grab (Fig. 2.4) with sampling area of 0.0675m^2. In the shallower sites, less than 20 m depth, divers collected cores using 150 mm PVC pipe corers to a depth of 100 mm which were then transferred to 0.875 mm mesh bags. These cores had a sampling area of 0.0177m^2. The diver core was the most reliable method in shallower water as samples of consistent depth could always be obtained, even in areas with coarse sediments. In deeper water the small Van-Veen grab was the best technique because it was heavy enough to provide consistent sample sizes, but light enough to be easily handled in a small boat. However, because the pilot study showed a significant difference in the number of species in samples collected by diver core compared with the small Van Veen grab ($P < 0.05$), this variable could not be compared between samples collected by these two methods.

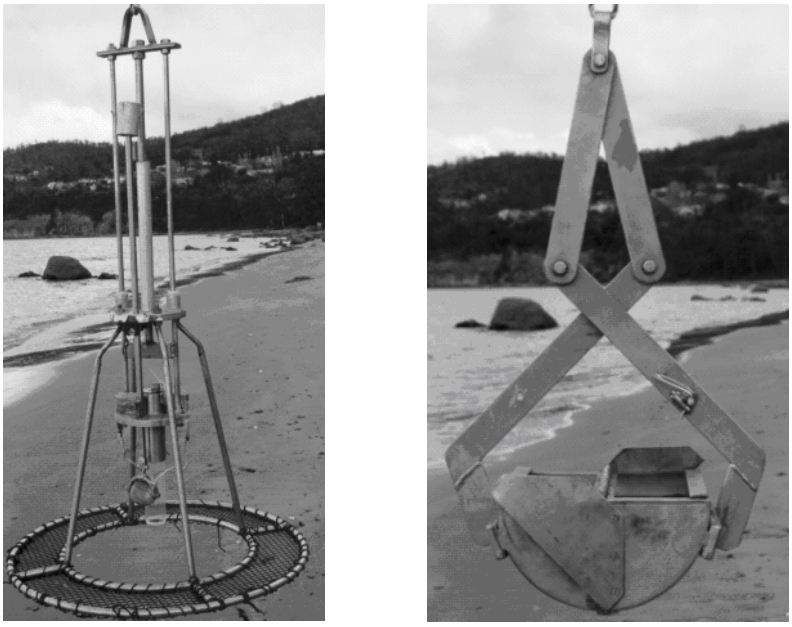


Fig. 2.4. Craib corer (left) and small Van Veen grab (right) used for collecting sediment samples.

Additional details of specific sampling techniques and analyses are provided in the following chapters.

3. Evaluation Of Physical And Chemical Parameters

3.1 Introduction

Physical and chemical measures of organic enrichment are included in many salmon farm monitoring programs in other countries. These variables regularly include total organic matter (from loss on ignition) and percentage organic carbon and percentage nitrogen. However, there are varying reports in the literature on the their reliability. In this report we investigate the suitability of these and several other environmental variables for monitoring the sediment around Atlantic salmon (*Salmo salar* L) farms. These assessments include using stable isotopes of C and N to trace the dispersion of organic matter from farms. To our knowledge, stable isotopes of C and N have not been previously used in aquaculture monitoring programs, although preliminary results from a salmon farm in Tasmania indicated potential (Ye *et al.*, 1990). Because a major objective of this research was to develop cost-effective methods for industry - wide routine monitoring of salmon farms, we avoided measures such as benthic respiration which require substantial time in the field. From the results obtained, we make some general recommendations on using physical and chemical variables for monitoring the effects of salmon farms on the benthic environment.

3.2 Methods

3.2.1 Sampling procedures

Triplicate sediment samples were collected at 0, 45 and 60 m sampling points along boundary transects, at one end of each reference transect, and at the farm cage transects (at the edge of cages F1, F2, F3 and 35 m from F3 at Nubeena; and at 0, 10, 35 and 60 m along the farm transect at Hideaway Bay). Samples were taken either remotely using the Craib corer in deeper water at Hideaway Bay, or by divers in shallow water using the internal core of the Craib corer.

Sediment cores collected using the Craib corer were examined within one hour of collection for colour using a Munsell Soil Colour Chart, evidence of a redox discontinuity layer, flora and fauna present, gas bubbles and smell (H_2S odour). The redox potential was measured and small quantities of sediment from the top 30 mm of the core were collected and frozen for later analysis of (i) total organic matter, (ii) sediment particle size, (iii) percentage organic carbon, (iv) percentage total nitrogen, and (v) stable isotopes of C and N.

3.2.2 Physical and chemical analyses

Sediment particle size composition was determined by wet sieving samples through 4, 2, 1 mm, 500 μm, 250 μm, 125 μm, and 63 μm mesh sieves, and measuring the volume of water displaced by sieve contents, except for the < 63 μm fraction which was calculated from the difference between initial and sum of the final volumes > 63 μm.

These values were expressed as a percentage of the volume of water displaced by the original sample before sieving.

Total organic matter (TOM) was determined by loss on ignition (Greiser and Faubel, 1988). A sub-sample of the top 3 cm of each core was oven dried at 60^0 C overnight, weighed and placed in a muffle furnace at 480^0 C for 2 hours, and then re-weighed. TOM was calculated from the difference between the oven dried weight and weight after being in the furnace, and was expressed as a percentage of the oven dried weight.

For $\%C_{org}$, %N and Stable Isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses, a sub-sample of the top 3 cm of each core was oven dried at 60^0 C, shell fragments were removed and samples were ground and mixed prior to the removal of 20-50 mg of sediment (weights varied according to the %TOM content of the sample). Samples analysed for $\delta^{13}C$ were first acidified with 0.1 M HCl for 24 - 48 hr until effervescence ceased, and then rinsed with distilled water to remove carbonates. A separate analysis was conducted for $\delta^{15}N$ on unacidified samples. The prepared samples were analysed by the CSIRO Land and Water Laboratories in Adelaide, South Australia for ^{12}C:^{13}C and ^{14}N:^{15}N stable isotope ratios, and $\%C_{org}$ and %N, using an in-line elemental analyser and mass spectrometer. Samples of pelleted fish food were also analysed. Triplicate samples were analysed from most sites, with duplicates from a few.

Stable isotope values were measured using the standard format

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = [(R_{sample} - R_{reference})/ R_{reference}) \times 100]$$

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$.

Due to funding constraints, $\%C_{org,}$ %N and stable isotope ratios were analysed at a sub-sample of transects and stations which are detailed in the results. However, at Hideaway Bay a greater number of boundary sites were sampled at 3 months as part of a baseline environmental assessment which was being conducted in accordance with Tasmanian State Government specification to obtain a licence to farm the lease area (Mitchell *et al.*, 1997).

Redox potential was measured using a WTW Microprocessor pH Meter with a combination Mettler Toledo pH/redox probe at the sediment surface, and at 1 cm intervals below the surface to a depth of 4 cm. The probe was calibrated between core readings using Zobells ferro/ferricyanide solution and allowed to equilibrate for 10 seconds before taking each reading. Results were corrected to the hydrogen reference probe.

3.2.3 Data analyses

Chemical data were analysed for significant differences ($P < 0.05$) between means by analysis of variance (ANOVA). Homogeneity of variances and normality of the data were examined from box plots, and some variables were transformed to normalise the data. Total organic matter was normalised using arcsine transformation, and redox

values were transformed using log (500-X). If significant differences were detected, then multiple comparisons were conducted using Tukeys test (Day and Quinn, 1989). Correlations between percentage organic matter and percentage sediment particle size <63 μm were assessed using the Pearson Correlation Coefficient (Zar, 1996) on arcsine transformed data.

Correlations between environmental variables and distance from the source of impact were tested using the Spearman Rank Correlation Coefficient (Zar, 1996). This assumes that the greatest impact occurs under the cage and progressively declines with distance from the cage. At Hideaway Bay, 8 ranks of distance from cages were used: 0 m, 10 m, 35 m and 60 m from a cage, 10 m inside lease boundary, 35 m and 50 m outside lease boundary, and reference sites. At Nubeena, 6 ranks of distance from cages were used: 0 m and 35 m from a cage, 10 m inside lease boundary, 35 m and 50 m outside lease boundary, and reference sites. A correlation was considered significant if $P < 0.05$.

3.3 Results

3.3.1 Sediment particle size

Sediments at the Hideaway Bay farm varied considerably over the lease area (Fig. 3.1). In the shallower inshore water the sediment was much coarser, e.g at R1 and B1 sites only 8% and 2-11%, respectively, of the sediment was silt and clays (< 63 μm). B2 sites were also relatively coarse with 14 - 32% silt and clays, compared to most of the other sites which had very high levels, ranging from 85 to 95% along transects B3-B6 (except at B3 60m). The reference site R4, furthermost out in the Huon River, had the highest silt and clay content of 97%. Along the farm transect from 0 to 60 m the sediment particle size < 63 μm varied from 94 - 54%, respectively. Sediment particle sizes were very similar between sites along transects B4, B5 and B6 that only transect B4 is shown in Fig. 3.1.

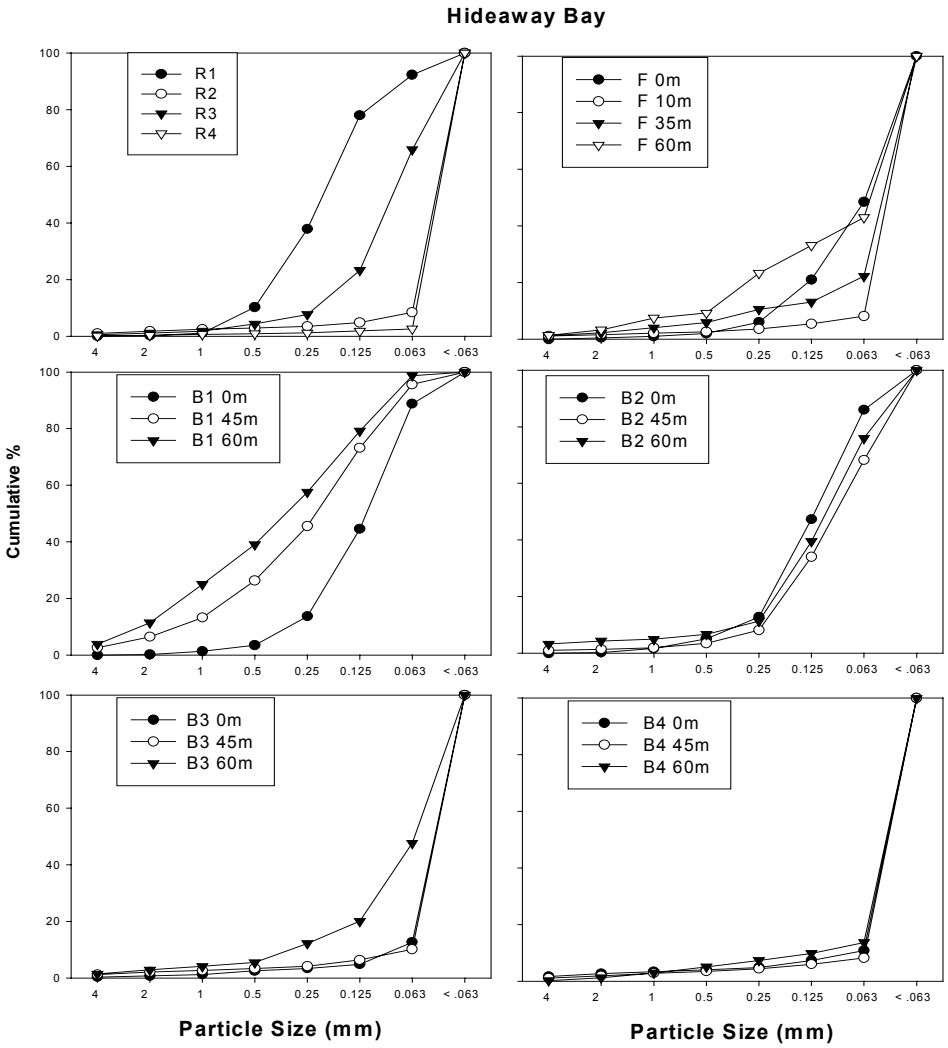


Fig. 3.1. Sediment particle size at Hideaway Bay farm, boundary and reference transect sites.

Sediment at Nubeena (Fig. 3.2) contained higher proportions of sand and shell fragments than at Hideaway Bay (Fig. 3.1). The sediment on the western side of the farm, (R1, B1 transects) was generally coarser than that at transects on the eastern side, (R2, B2 transects), and there was a transition towards finer sediment across the farm from west to east. Mean % silt and clays (< 63 μm) at the western sites ranged from 0.8 to 8% and at the eastern sites from 12.5 - 23%.

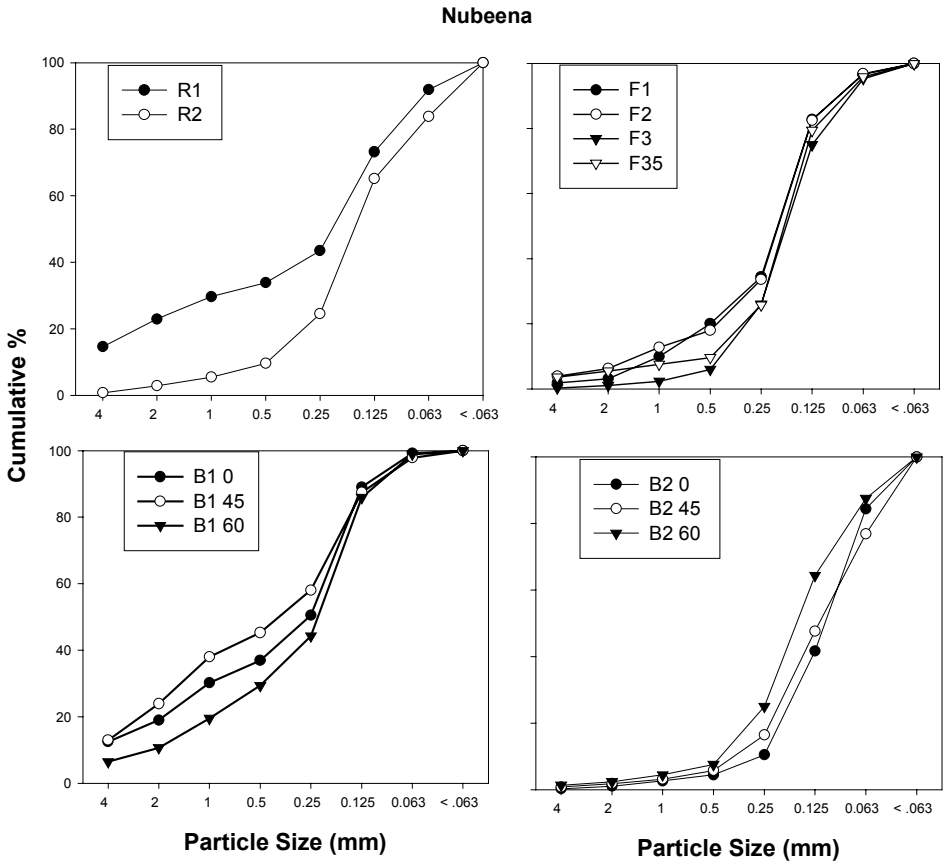


Fig. 3.2. Sediment particle size at Nubeena farm, boundary and reference transect sites.

3.3.2 Percentage total organic matter (TOM)

At Hideaway Bay, TOM was significantly greater ($P < 0.001$) at transects containing high proportions of silts and clays (transects R2-R4, B3-B6, F) than at transects with coarser sediments (sites R1, B1-B2) closer to shore (Fig. 3.3). Correlation analysis showed a highly significant correlation between TOM and % particle size < 63 μm, ($r = 0.816$, $P < 0.01$, $n = 26$). Background levels of organic matter in the Huon River were also high, 16-18%, at reference sites with fine sediment particle size.

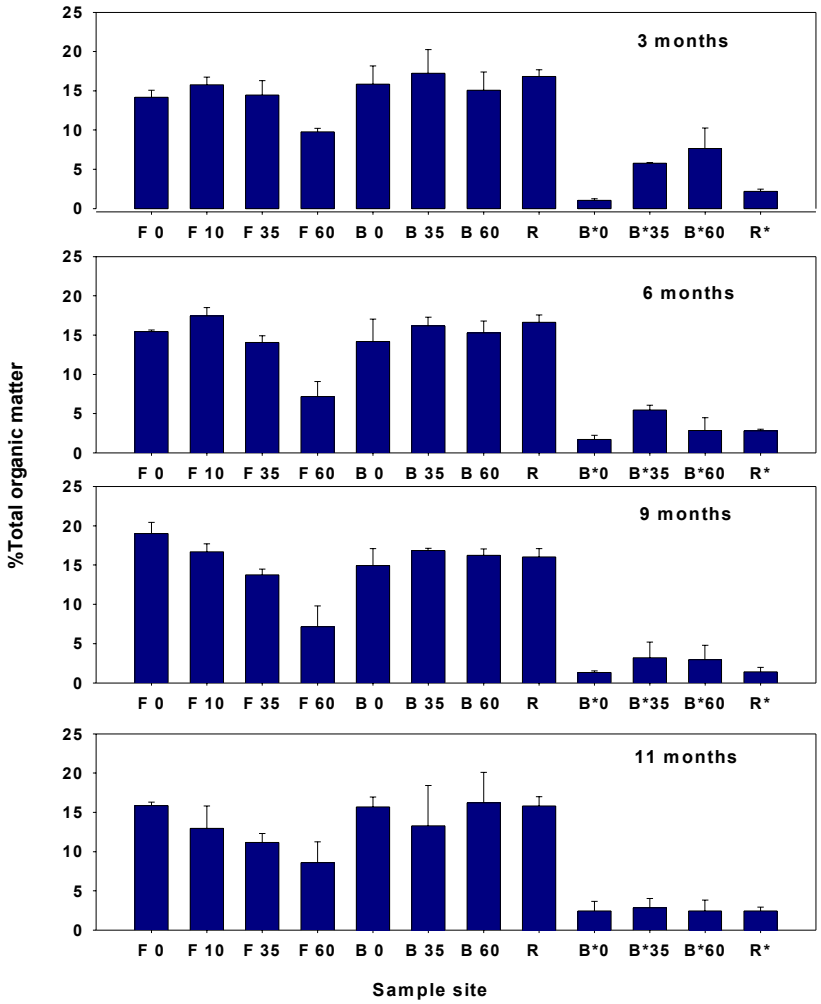


Fig. 3.3. Mean Percentage Total Organic Matter at Hideaway Bay, at farm cage sites F 0 - 60 m, at the boundary transects B3 – B6 and reference sites R2 – R4 with finer sediment (shown as B 0, B 35, B 60, R), and at the inshore boundary transects B1 – B2 and reference site R1 with coarser sediment (shown as B*0, B*35 and B*60, R*).

TOM values along the farm cage transect were greatest at 0 and 10 m from the cage edge by the end of the survey period (Fig. 3.3), and progressively decreased with further distance from the cage. However, ANOVA showed that these high values at 0 and 10 m from the cage were not significantly different from other values recorded outside the lease area in deeper water, including reference sites ($P > 0.05$).

In order to account for the correlation between organic matter and sediment particle size, the differences between predicted TOM as would be expected at an unpolluted site of similar grain size, and the measured TOM values around the farm were compared. From a study of reference sites considered to be unaffected by farming and adjacent to 23 farming sites in south eastern Tasmania, the general relationship between organic matter and sediment particle size has been calculated as:

$$TOM = 1.85 + 0.229X,$$

where X = sediment particle size (G. Edgar, unpublished data 2000).

This equation was used to predict levels of TOM that would be expected at the farm site if no organic enrichment occurred, and the difference between the predicted and observed TOM values (residuals) were calculated. Analysis of variance using these residual data showed no significant differences between farm and reference sites ($P>0.05$). The correlation between residual TOM and distance from the source of impact at four different sampling times also was not significant (Table 3.1).

Table 3.1. Spearman Rank Correlation Coefficient between environmental variables and distance from salmon cage.

Variable	Time (month)	n	r_s	P
Hideaway Bay				
TOM	3	25	-0.269	P > 0.05
	6	26	-0.206	P > 0.05
	9	26	-0.219	P > 0.05
	11	26	-0.191	P > 0.05
Redox	6	26	0.512	P < 0.01
	9	26	0.425	P < 0.05
	11	26	0.524	P < 0.01
%N	6	9	-0.542	P > 0.05
	11	9	-0.374	P > 0.05
%C	6	9	-0.542	P > 0.05
	11	9	-0.203	P > 0.05
$\delta^{15}N$	6	9	-0.871	P < 0.01
	11	9	-0.814	P < 0.01
$\delta^{13}C$	6	9	-0.850	P < 0.01
	11	9	-0.373	P > 0.05
Nubeena				
TOM	5	12	0.362	P > 0.05
	10	12	0.096	P > 0.05
Redox	5	12	0.082	P > 0.05
	10	12	0.028	P > 0.05

At Nubeena TOM values (range 1 - 8%) were lower than at Hideaway Bay, but showed similar trends (Fig. 3.4). Two-way ANOVA of arcsine transformed %TOM at reference sites, transects B1 and B2 at 35 m from the farm boundary and at the farm sites at the 0 and 5 month samplings showed a significant difference between transects, but not over time, and no significant interaction of transect x time. The percentage TOM was significantly higher at the reference sites and eastern transect B2 than at the farm sites and western boundary B1.

A highly significant correlation ($r = 0.758$, $P < 0.01$, $n = 12$) was found between the proportion of silt/clays (percentage sediment particle size < 63 μm) and percentage organic matter. Using residual TOM values, no significant differences ($P > 0.05$) were detected between sites next to cages, at the boundary and at reference sites. Similarly, the correlation between residual TOM and distance from cages was not significant (Table 3.1).

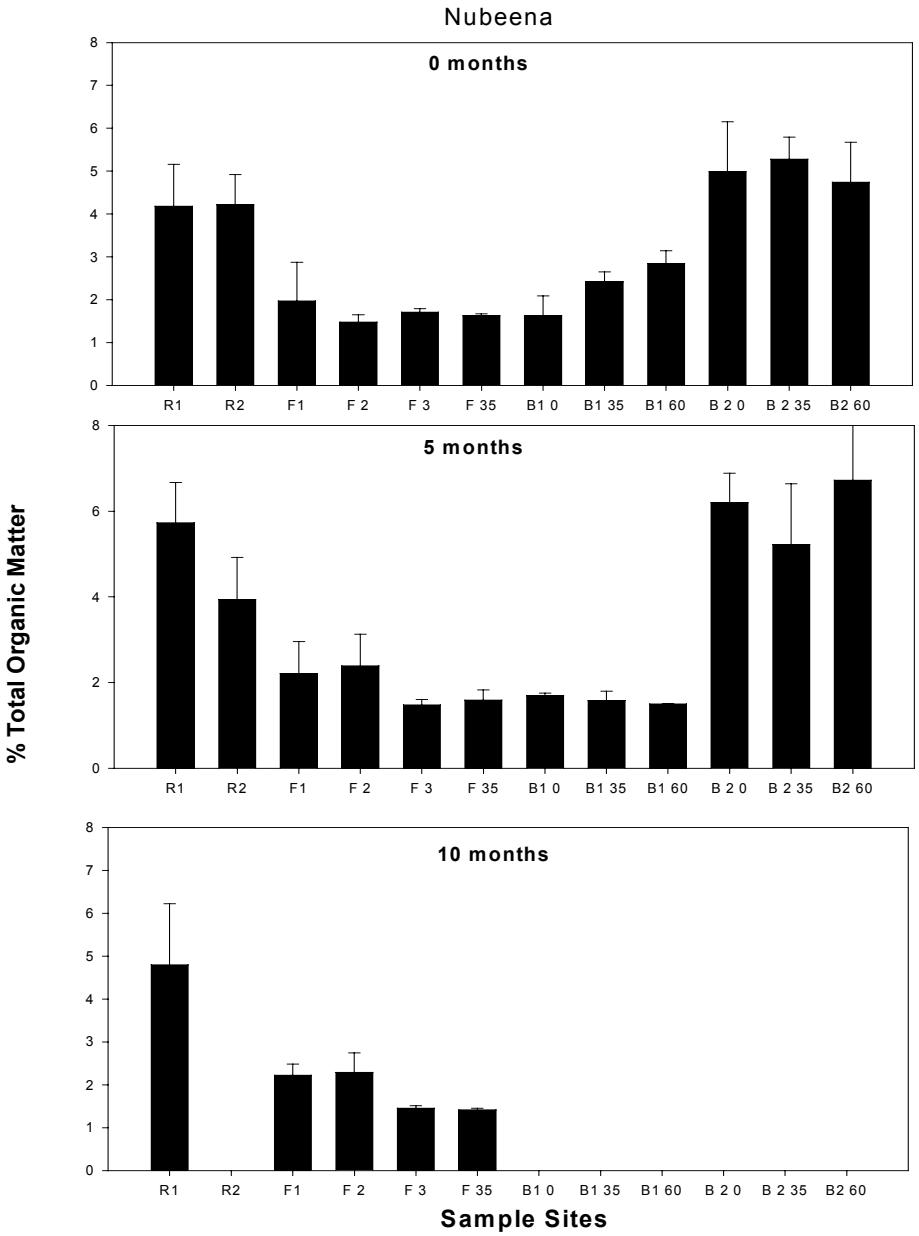


Fig. 3.4. Percentage total organic matter at the Nubeena farm sites.

3.3.3 Percentage nitrogen and organic carbon

Pelleted salmon feed of length 8 - 9.25 mm and protein/carbohydrate ratio of 45/25 to 45/30 contained relatively high amounts of nitrogen (8.13% ± 0.47) and carbon (45.35% ± 1.50), with an average C:N ratio of 5.6 ± 0.41. Two samples of fish faeces contained 3.9 - 4.9 %N and 31.8 - 40.3 %C (McGhie *et al.*, 2000).

Percentages of organic carbon and nitrogen, and stable isotopes, were only evaluated at selected reference, boundary and farm sites at the 6 and 11 month samplings at Hideaway Bay and at 0, 5 and 10 months at Nubeena because of funding constraints. Additional data are also available from Hideaway Bay at 3 months from a separate baseline environmental assessment. The percentages of organic carbon and nitrogen

were generally 4-5 times higher at the Hideaway Bay farm than at Nubeena (Table 3.2 and Table 3.3). At Hideaway Bay %N and $\%C_{org}$ were highest next to the farm cage (F 0 m) and approximately twice the level of boundary transect and reference sites at the 6 month sampling (Fig. 3.5, Table 3.2). F 10 m also had elevated nitrogen levels at the 6 month sampling, and F 0 m at 11 months. Reference and boundary transects sites had similar %N (approximately 0.4%) and $\%C_{org}$ (approximately 5.2 - 6.0 %) at the 6 and 11 month samplings, except for R1. Nevertheless, there was not a significant correlation between either %N or $\%C_{org}$ and distance from the source of impact, largely because of variability across the farm, and generally lower values at sites with coarser sediments. (Table 3.1).

A comparison of %N and $\%C_{org}$ values after 3 months (Table 3.2) at farm boundary transects B1, B2, B3, B5 and reference sites R1, R2 and R4 indicated two main grouping: B1, B2 and R1 in shallow water with coarse substrate (range %N 0.1 - 0.3, $\%C_{org}$ 0.2 - 3.7), and a grouping of B3, B5, R3 and R4 in deeper water with predominantly silt/clay sediments (range %N 0.2 - 0.5, $\%C_{org}$ 5.6 - 6.1).

Table 3.2. %N, $\%C_{org}$, $\delta^{15}N$ and $\delta^{13}C$, and C/N ratio at Hideaway Bay. s.d.= standard deviation

Site	Month	%N	s.d.	$\delta^{15}N$	s.d.	$\%C_{org}$	s.d.	$\delta^{13}C$	s.d.	C/N
R1	3	0.14	0.07	7.42	0.24	0.39	0.17	-23.72	0.28	2.74
R3	3	0.42	0.03	7.46	0.21	5.64	0.14	-22.77	0.05	13.34
R4	3	0.43	0.01	7.71	0.13	5.79	0.09	-23.11	0.06	13.61
B1 0 m	3	0.13	0.10	7.57	0.38	0.21	0.03	-23.40	0.06	1.62
B1 35 m	3	0.15	0.01	7.56	0.33	1.38	0.33	-23.26	0.27	9.02
B1 60 m	3	0.18	0.03	7.81	0.44	1.47	0.29	-22.38	2.17	8.19
B2 0 m	3	0.12	0.08	7.91	0.14	0.30	0.03	-23.85	0.37	2.41
B2 35 m	3	0.16	0.05	7.79	0.32	1.27	0.29	-23.44	0.04	7.76
B2 60 m	3	0.30	0.14	7.65	0.24	3.68	0.23	-23.10	0.04	12.14
B3 0 m	3	0.42	0.05	8.02	0.19	5.91	0.06	-22.98	0.03	14.16
B3 35 m	3	0.23	0.16	7.32	0.09	5.76	0.10	-22.99	0.04	25.03
B3 60 m	3	0.28	0.15	7.45	0.37	5.76	0.18	-23.05	0.02	20.29
B5 0 m	3	0.43	0.01	7.78	0.29	5.71	0.19	-22.96	0.03	13.20
B5 35 m	3	0.44	0.01	7.90	0.35	5.90	0.14	-22.95	0.09	13.50
B5 60 m	3	0.45	0.02	7.85	0.23	6.08	0.10	-22.81	0.05	13.61
R1	6	0.03	0.01	5.29	0.37	0.36	0.13	-24.00	0.29	10.80
R3	6	0.40	0.01	7.36	0.10	5.65	0.22	-22.97	0.09	14.00
R4	6	0.42	0.01	7.04	0.06	5.36	0.78	-23.11	0.07	12.86
F 0 m	6	0.94	0.12	10.96	0.40	8.84	0.54	-20.11	0.29	9.37
F 10 m	6	0.65	0.11	9.76	0.24	7.25	1.07	-21.93	0.31	11.11
F 35 m	6	0.37	0.01	8.16	0.12	5.34	0.39	-22.59	0.21	14.55
F 60 m	6	0.19	0.05	8.45	1.25	2.86	0.75	-13.98	2.06	15.07
B4 0 m	6	0.44	0.00	7.28	0.19	6.08	0.22	-22.82	0.08	13.82
B4 35 m	6	0.43	0.01	7.18	0.03	5.95	0.28	-22.83	0.03	13.74
R1	11	0.08	0.04	6.16	0.83	0.38	0.06	-24.02	0.01	4.69
R3	11	0.40	0.01	7.41	0.09	5.52	0.27	-22.75	0.10	13.69
R4	11	0.42	0.01	7.27	0.17	5.89	0.06	-23.07	0.05	13.92
F 0 m	11	0.52	0.05	8.05	0.23	6.50	0.16	-22.39	0.37	12.42
F 10 m	11	0.42	0.02	7.91	0.18	5.50	0.29	-23.01	0.13	13.08
F 35 m	11	0.28	0.04	7.72	0.16	3.64	0.49	-22.95	0.08	13.01
F 60 m	11	0.23	0.08	7.74	0.21	3.24	1.71	-20.44	2.99	14.09
B4 0 m	11	0.45	0.02	7.09	0.14	6.00	0.01	-22.90	0.06	13.22
B4 35 m	11	0.44	0.01	7.35	0.02	6.07	0.02	-22.77	0.04	13.89

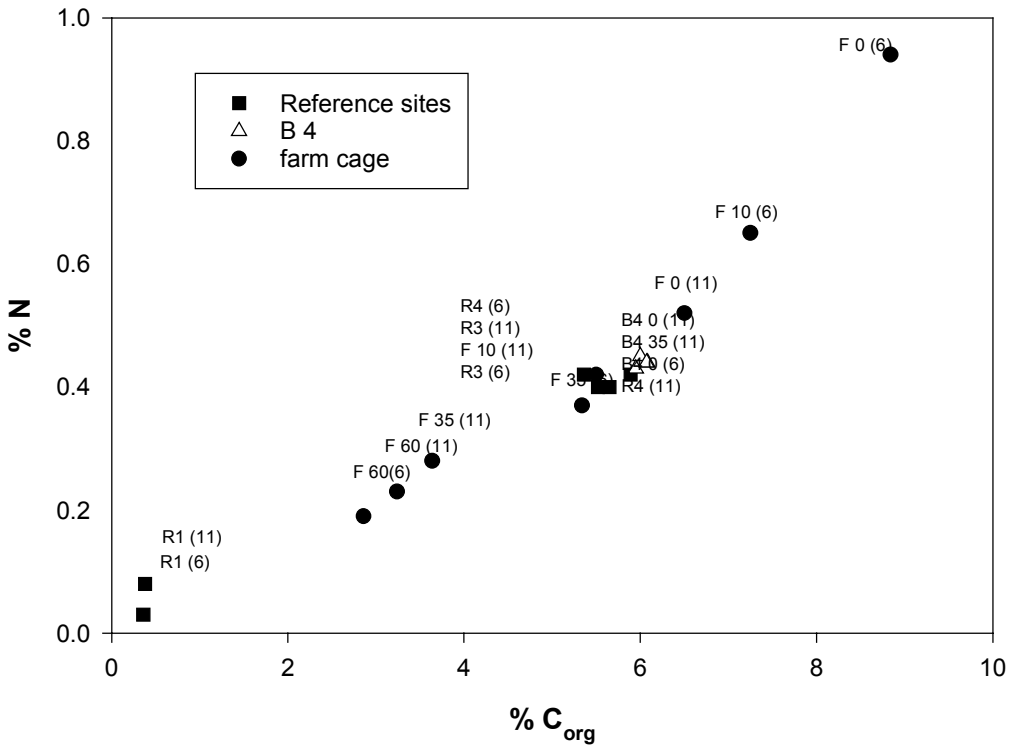


Fig. 3.5. $\%C_{org}$ and %N at Hideaway Bay farm boundary, reference and farm transect sites at the 6 and 11 month surveys.

%N at the Nubeena farm was low (0.04 - 0.19%) and typical of a marine site with predominantly sand substrate type (Table 3.3). The reference sites had higher %N levels than the farm sites, except for F2 after 5 months. For example, at the 10 month survey, %N at R1, F2, F3 and F 35 was 0.19, 0.13, 0.06 and 0.07, respectively. Similarly, $\%C_{org}$ was higher at the reference and boundary sites than next to the salmon cages at Nubeena. Ranges in $\%C_{org}$ over the three sampling periods at Nubeena were 0.71 - 1.87 at reference sites, 0.32 - 0.54 at F cage sites, and 0.46 - 1.02 at boundary transects.

Table 3.3. %N, $\%C_{org}$, $\delta^{15}N$ and $\delta^{13}C$, and C/N ratio at Nubeena. s.d.= standard deviation

Site	Month	%N	s.d.	$\delta^{15}N$	s.d.	$\%C_{org}$	s.d.	$\delta^{13}C$	s.d.	C/N
R2	0	0.11	0.03	6.44	0.41	0.71	0.21	-23.45	0.37	6.41
R1	0	0.15	0.06	6.84	0.82					
F2	0	0.04	0.01	6.69	0.93					
F3	0	0.06	0.03	6.59	0.68					
F4	0	0.04	0.03	6.06	0.86					
R2	5			6.00	0.19	0.94	0.53	-23.20	0.32	
R1	5	0.15	0.07	6.11	0.10	1.52	0.85	-23.20	0.04	10.11
F2	5	0.19	0.04	7.88	0.21					
F3	5	0.07	0.01	7.48	0.57	0.54	0.04	-23.34	0.32	7.41
F4	5	0.10	0.05	7.12	0.27	0.53	0.09	-22.97	0.40	5.33
B1 0 m	5	0.05	0.02	7.01	0.21	0.46	0.23	-23.22	0.13	9.79
B1 35 m	5	0.10	0.08	7.07	0.23	1.02	0.88	-23.15	0.23	10.20
R1	10	0.19	0.04	6.99	0.12	1.87	0.69	-23.08	0.17	10.11
F2	10	0.13	0.05	7.76	0.93					
F3	10	0.06	0.01	6.36	0.11	0.32	0.00	-23.28	0.19	5.82
F4	10	0.07	0.01	7.23	0.71	0.46	0.09	-22.91	0.28	6.95

3.3.4 Stable isotopes

Stable isotope values of the three types of salmon feed pellets ranged from 11.50 to 13.59 ‰ for $\delta^{15}N$ and -19.80 to -20.96 ‰ for $\delta^{13}C$. Values for faeces (from McGhie *et al.*, 2000) varied from 9.6 to 12 ‰ for $\delta^{15}N$ and -18.1 to -17.4 ‰ for $\delta^{13}C$.

At Hideaway Bay the farm cage transect sites (F's) from next to the cage to 60 m away had elevated $\delta^{13}C$ values compared to reference sites and boundary transect sites (Table 3.2, Fig. 3.6). In particular, $\delta^{15}N$ values at F 0 m and F 10 m after 6 months were closer to the fish food values than to values for other sites. $\delta^{13}C$ was lowest at R1 and between –22 ‰ and –23 ‰ at most other sites. Exceptionally high values of $\delta^{13}C$ at F 60 m at 6 months were possibly caused by problems associated with analytical methods. The carbonate in the sediment may not have been completely removed by acidification.

The correlation between $\delta^{15}N$ and distance from the fish cage at Hideaway Bay was significant ($P < 0.01$) at both the 6 and 11 month surveys (Table 3.1). $\delta^{13}C$ was significantly correlated with the source of impact at 6 months, but not at the 11 month survey (Table 3.1).

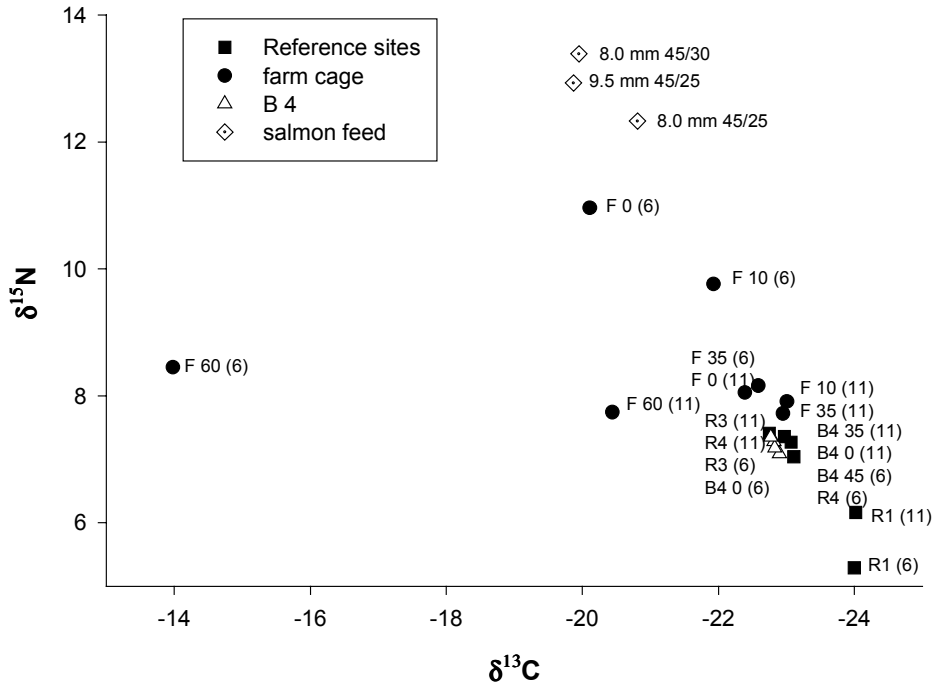


Fig. 3.6. Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ values at selected sites at the Hideaway Bay salmon farm, and of fish food pellets.

Stable isotope ratios at farm boundary transects B1, B2, B3, and B5 at 3 months (Table 3.2) showed little variation between sites. $\delta^{15}N$ values ranged from 7.4 to 8 $^{0}/_{00}$ and the $\delta^{13}C$ values ranged from -23.9 to -23.0 $^{0}/_{00}$. These values were very similar to those for the group B4, R3, and R4 in Fig. 3.6.

$\delta^{13}C$ values at Nubeena were very similar at all sites and varied by less than 0.6 $^{0}/_{00}$ between farm and reference sites (Table 3.3). Lowest values were at R2 at the first sampling (0 months), and highest at F4 after 5 and 10 months.

Mean $\delta^{15}N$ values ranged from 6 to 8 $^{0}/_{00}$ which are considerably lower than the fish food. Only at the 5 month sampling were the $\delta^{15}N$ values higher at the farm than reference sites; at the 0 and 10 month samplings there were no clear patterns between reference and farm sites.

3.3.5 Redox

The redox data below the surface, particularly at the deeper depths, are missing some measurements because redox was only measured until the 0 value was reached on some occasions. Also the redox probe would not penetrate some sediment cores, particularly coarse sandy substrates. Consequently, only redox data from 1 cm below the surface were analysed statistically, even though Pearson and Stanley (1979) analysed data obtained at 4 cm because they found that Eh values were more stable at this depth and generally representative of the overall values down the sediment column. Our results also show the greatest and most rapid changes in redox occur at the sediment surface.

At Hideaway Bay redox values at 1 cm depth differed significantly ($P < 0.01$) between sites at each sampling time 6, 9 and 11 months (Fig. 3.7). Redox was not measured during the 3 month survey because of a malfunctioning probe. Lowest values were recorded at sites close to the salmon cage. Only these farm sites, F 0 m and F 10 m, reached negative redox values at both 1 cm and 4 cm depth at the 6 month sampling. Redox values at F 0 m were still low after 9 months, but were similar to other sites at 1 and 4 cm depth at 11 months. Redox values also showed a significant correlation with distance from salmon cage on each sampling occasion (Table 3.1).

Similarly, redox values at Nubeena were generally lower at the edge of the farm cages than at boundary and reference sites (Fig. 3.8). Values below zero were only recorded at farm sites. At F2, even surface values were below zero at the 5 month sampling, but these had substantially improved by 10 months when all readings at F2 were above zero. However, redox values at 4 cm depth at F3 remained negative at both the 5 and 10 month samplings, suggesting a slower rate of recovery at this site. The correlation between redox and distance from a cage was not significant (Table 3.1).

At 1 cm depth at Nubeena redox values were significantly different ($P < 0.001$) between sites but not between seasons. Tukeys test showed that redox was significantly higher at F35 and B1 45 than at F1, F2 and R1.

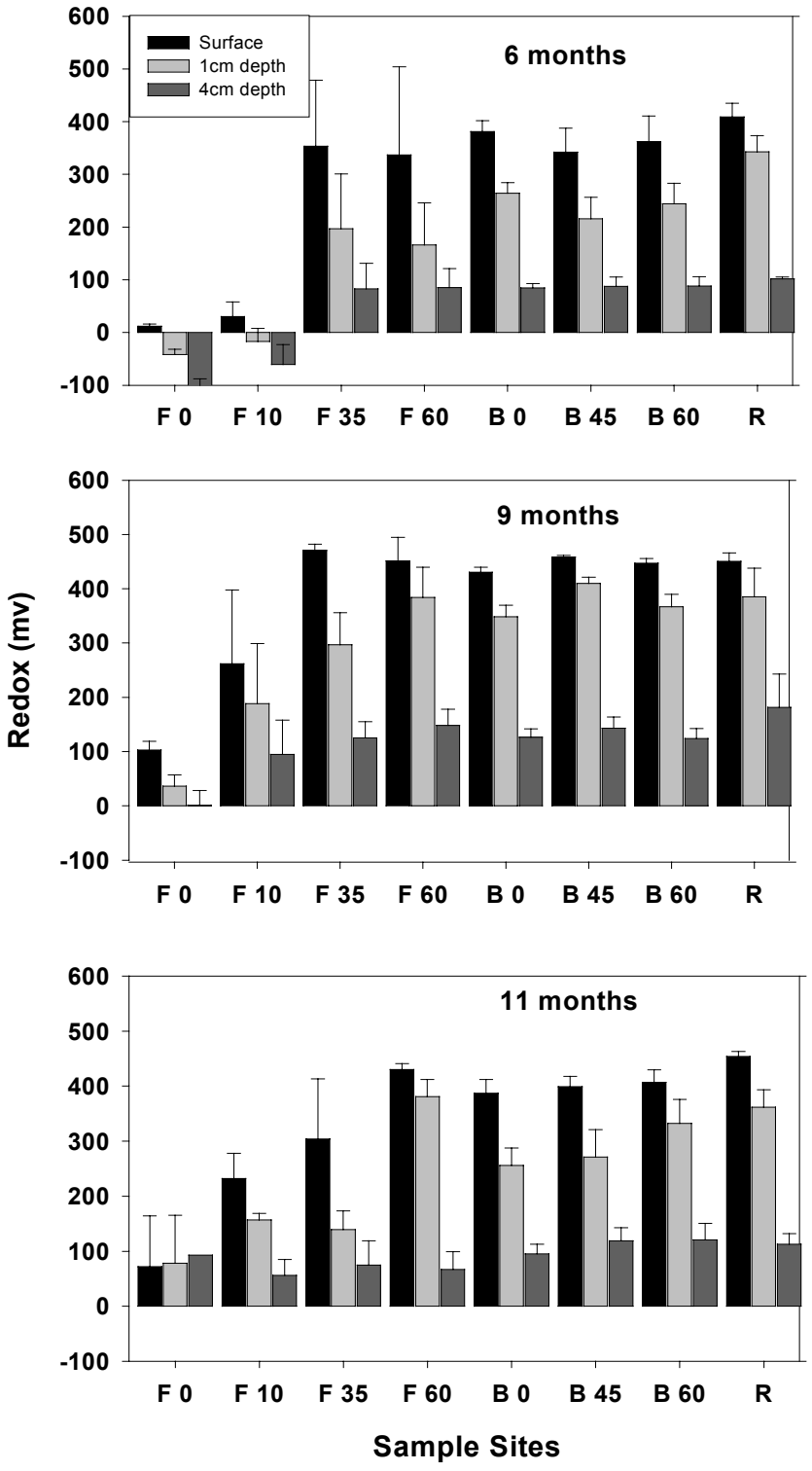


Fig. 3.7. Redox potential at Hideaway Bay stations at 6, 9 and 11 month sampling times.

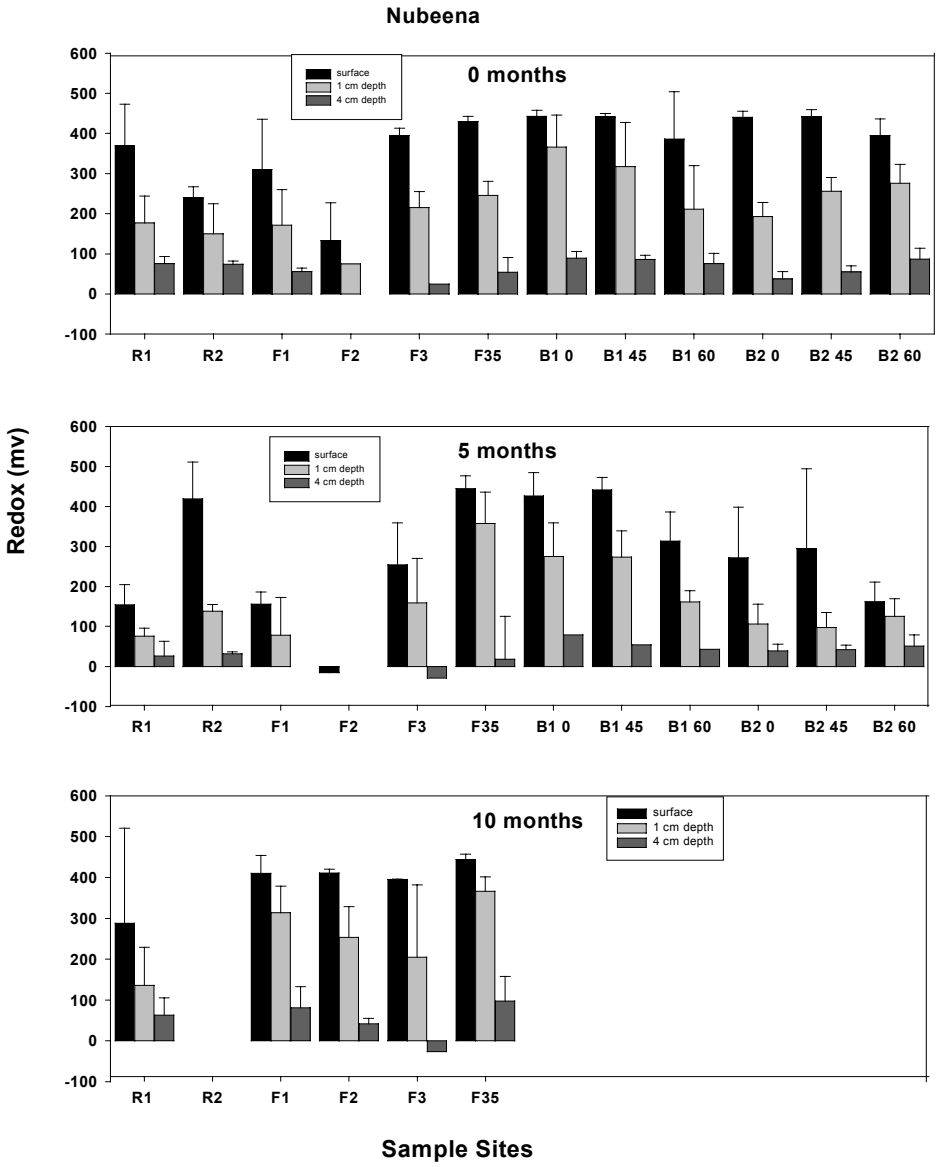


Fig. 3.8. Redox potential at Nubeena sites at 0, 5 and 10 month sampling times.

At many sites redox values were highest at the 3 and 9 month sampling times (summer and winter), lowest after 6 months (autumn), and more variable and at intermediate levels after 11 months (spring). Examples of redox values at several sites over time are shown in Fig. 3.9. This suggests a possible seasonal change in redox, however, further sampling over a longer period of time would be required to validate seasonal changes.

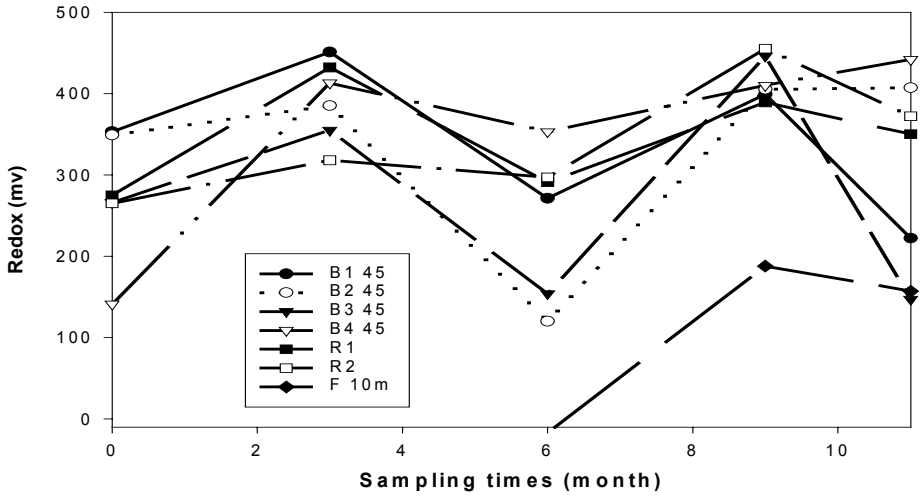


Fig. 3.9. Redox values at 1 cm depth at sites R1, R2, F 10 m, B1 45 m, B2 45 m, B3 45 m, and B4 45 m at four sampling times during the year.

3.4 Discussion

Our results for percentage total organic matter at Nubeena are different to those recorded by Ye *et al.* (1990) at the same site. They found much higher values directly under the cage (5 – 9 % in the top 2 cm) than at 10 - 150 m away (2 %), whereas we consistently recorded significantly higher values of organic matter at both reference sites and the eastern transect sites than next to the farm cages. However, Ye *et al.* (1990) only collected samples along one transect from the cage and did not sample at reference sites. Although it is possible that organic wastes could have been accumulating at the reference sites, this was not obvious in the video recordings of these transects (Crawford *et al.*, 2001). The video footage showed that at the 5 month sampling the reference sites had abundant macroalgae and fauna, whereas the farm transect next to cages was obviously impacted with dense bacterial mats and accumulations of pellets and fish faeces clearly visible on the bottom.

Similarly, at Hideaway Bay %TOM was higher at several reference and boundary transect sites than next to the farm cage, and this pattern differed from other environmental parameters which clearly showed cage sites to be impacted (Crawford *et al.*, 2001).

These results imply that organic matter, as measured by loss on ignition, is not a reliable measure of degradation at salmon farm sites. Several researchers have found that organic matter measurements by loss on ignition were accurate only if the sediments were low in carbonates or clays. The breakdown of relatively high levels of carbonates can interfere with the ratio of organic to inorganic carbon (Byers *et al.*, 1978; Kristensen and Anderson, 1987; Nieuwenhuize *et al.*, 1994), whereas large amounts of clay in the sediment may result in overestimates of organic matter because of loss of structural water during ignition (Byers *et al.*, 1978; Craft *et al.*, 1991). As the majority of our sediment samples contained relatively high levels of both carbonates and clays, it is possible that some of our results may not be reliable.

The significant correlation between sediment particle size < 63 μm and TOM that we found has also been documented by Parsons *et al.* (1977), and was found to reflect the increased surface area for organic adsorption in small sized silts and clays. Although this correlation is generally well known amongst marine benthic ecologists, it appears to have rarely been taken into consideration in salmonid environmental monitoring programs.

Results of $\%C_{org}$ and %N had many similarities to TOM. %N and $\%C_{org}$ values were generally much higher at the Hideaway Bay farm than at Nubeena. Even so, they were much lower at both farms than for the fish food and faeces.

At Nubeena $\%C_{org}$ and %N of the sediments do not show a clear correlation with the degree of impact of the fish farm because they were generally similar or higher at the reference sites than at the farm sites.

By contrast, at Hideaway Bay %N and $\%C_{org}$ were higher next to the farm cage than at reference and boundary sites at the 6 month sampling period, and to a lesser extent at 11 months. These results contrast with those for percentage organic matter, as measured by loss on ignition, which did not increase at the farm site even when impact on the bottom was major as shown by other environmental variables. Other environmental indicators also suggested high organic loading at these times (Crawford *et al.*, 2001). Nevertheless, %N and $\%C_{org}$ at sites with a relatively coarse substrate, e.g. R1, were much lower than at other reference and boundary sites with finer substrate characteristics. These results suggest that $\%C_{org}$ and %N are suitable indicators of organic matter only when there are very high levels of organic deposition. However, similar to measurements of organic matter by loss on ignition, sediment particle size may be an important factor in determining organic carbon and nitrogen concentrations at lower levels of organic enrichment.

Organic matter as measured by loss on ignition, and total organic carbon measured using a CHN elemental analyser have been widely used in environmental monitoring programs. However, several other researchers have also questioned the suitability of these measures in salmon farm monitoring programs. For example, Henderson and Ross (1995) in Scotland observed marked variation in organic carbon content at 23 fish farms and found no consistent patterns with distance from cages, site characteristics or maximum biomass of fish in the cages. At a gilthead sea bream farm in Greece where the water currents were low and the substrate silty, Karakassis *et al.* (1998) found that the organic matter was significantly higher under the cage than at all other sites but was not different between sites 5 m, 10 m, and 25 m from the cage and a reference site. They concluded that LOI and TOC were good estimators of organic carbon only in very highly enriched environments and that generally they were a "poor descriptor of the farm impact".

Stable isotope values also only clearly identified the effects of organic enrichment at times when other environmental variables also indicated very high levels of organic enrichment. At Nubeena the $\delta^{15}N$ values were higher at farm sites than at reference site R2 only when the video showed a severe impact under the cages, and although pellets could be seen on the bottom, the $\delta^{15}N$ values were still substantially below those for food pellets.

Results for $\delta^{13}C$ from Nubeena differ from those of Ye *et al.* (1990) even though they were obtained from the same farm, but approximately 9 years later. Our $\delta^{13}C$ values were lower (1 – 2 ‰) under the fish cage, and whilst our values did not change significantly between sites within the farm, their results increased with distance from the cage for 60 m. Also, the background values of -13.03 ‰ in a sediment trap under the cage and -19.79 ‰ for the sediment recorded by Ye *et al.* (1990) were considerably higher than our reference site values of approximately –23 ‰. These differences in results may be influenced by other sources of organic carbon, for example $\delta^{13}C$ of seagrass which occurs in the area was -10.88 ‰ (Ye *et al.*, 1990).

At Hideaway Bay only the $\delta^{15}N$ levels sampled after 6 months in autumn were clearly higher next to the cage than at the reference and other sites, and approached the values obtained for the fish food. The benthic infauna, sediment redox values and video records around the cage also indicated heavy levels of organic enrichment at that time (Crawford *et al.*, 2001). However, after 11 months $\delta^{15}N$ was only marginally higher at the farm cage than most other sites. These results obtained along the farm transect indicate that at times of high deposition of organic wastes from a fish cage, $\delta^{15}N$ values are highest next to the cage and become lighter with distance from the cage to 35 m. By contrast, Hansen *et al.* (1990) found that $\delta^{13}C$ values remained relatively constant at sites along a transect which received high organic loading, but progressively returned to background levels along a 30 m transect at sites with low accumulation of wastes. They suggest that the higher sedimentation rates probably impact a larger area of the farm, and this in combination with reduced secondary conversion by infauna may have resulted in the relatively constant isotope values along the transects.

The $\delta^{13}C$ values at the Hideaway Bay farm cage sites were highly variable compared to the reference and farm boundary sites. At the 6 month sampling the $\delta^{13}C$ value next to the cage was similar to that of fish food, suggesting a significant build up of food wastes. The high $\delta^{13}C$ values at 60 m from the cage, especially after 6 months, suggest other sources of carbon or high carbonate levels in the samples have affected measurements of $\delta^{13}C$. Midwood and Boutton (1998) found that treatment of calcareous soils with 0.1 M HCl for less than three days did not remove all the carbonate, resulting in significantly higher $\delta^{13}C$ values. Thus the high $\delta^{13}C$ values at this site may have occurred because not all the carbonate was removed. However, this does not explain why R1, which has a coarser substrate type relative to all the other sites, had $\delta^{13}C$ values which were significantly lower than the other sites. These anomalies in the $\delta^{13}C$ results suggest that the current technique is inadequate and better methods for acidification of samples are required.

The results from this study and those from an investigation of the rate of recovery of fallowing sites at Hideaway Bay (McGhie *et al.*, 2000), suggest that the effects of organic enrichment on the sediment from the cage of fish are mostly localised (in the cage shadow), and that any farm wastes which are more widely dispersed are masked by the dominant characteristics of the sediment with which they are mixed. Similarly, Thornton and McManus (1994) suggested that point source sewage discharge had limited influence on the isotopic composition of the sedimentary particulate organic matter (POM) because of the overwhelming volume of estuarine POM. It is also likely that the increased nitrogenous fish farm wastes would stimulate increased faunal, floral

and bacterial activity which would alter the isotopic signature, as suggested by Thornton and McManus (1994) for sewage effluent.

Overall, $\delta^{13}C$ values either varied very little between reference and farm impacted sites, or else the results were anomalous, and indicate that further research is required to standardise methods and better understand the ecological processes occurring in the vicinity of the fish farms. Also, $\delta^{15}N$ appears to be a useful tracer of salmon farm wastes only when organic enrichment is high and other environmental parameters also indicate severe impact. The cost-effectiveness of measuring $\delta^{15}N$ values and the lack of sensitivity of this procedure needs to be evaluated against the benefits of other measures of organic enrichment.

Redox values measured around the two farms showed some variation between replicates and between sites but, nevertheless, provided a clear distinction between redox values measured at the boundary sites and those measured at relatively degraded farm sites. These results suggest that a negative redox result, which indicates anoxic conditions, at both 1 and 4 cm depths is a useful measure of degraded conditions. Measuring redox has the advantage that it is relatively quick and inexpensive compared to other environmental parameters. However, standard procedures must be followed to obtain reliable and accurate results. Also, from our experience, redox probes need to be regularly calibrated and replaced because subtle drifts in results can occur with regular use over 6 – 12 months.

Researchers differ in opinions on the suitability of redox for monitoring environmental change around fish farms. Studies in Scotland showed wide variability in redox values between farm sites, and a non-consistent pattern with organic enrichment or benthic infauna data (Henderson and Ross, 1995). This variation was largely attributed to site-specific parameters and Henderson and Ross (1995) suggested that environmental degradation of a site would be better inferred from a site specific time series of measurements than generalised ratings of levels of impact across all sites. Because of this variability, Henderson and Ross (1995) were unable to recommend environmental quality standards for redox or organic carbon, nor could they recommend that redox or organic carbon were a suitable surrogate for benthic biological data. By contrast, Pearson and Stanley (1979) concluded that redox potential was a good rapid means of assessing the impact of organic enrichment from paper and pulp mill effluent. At both a gilthead sea bream farm in Greece (Karakassis *et al.*, 1998) and a red sea bream farm in Japan (Tsutsumi, 1995), the redox values were lowest under the cages and increased with distance from the farming area. In the Bay of Fundy salmon growing area in Canada a combination of redox and sulphide measurements is currently being recommended for monitoring of salmon farms (Wildish *et al.*, 1999).

In the present study the results for redox, stable isotopes and percentage organic carbon and nitrogen from both farms indicated that the effect of organic enrichment was greatest in autumn, although additional data collected over at least another full year would be required to verify a seasonal effect. The results obtained in autumn show the effects of increasing water temperatures from spring to summer and higher rates of metabolism in the fish, resulting in increased feeding rates. These results, albeit preliminary, suggest that autumn is the best time for monitoring the effects of fish farms on the environment. In other salmon producing countries greater impact in autumn has

also been observed and monitoring has been recommended at this time (e.g. Maine; (Heinig, 1996)).

The results from this study of two salmon farms show that natural variability in environmental variables across a farm, such as sediment particle size, can have a major effect on the adsorption and accumulation of organic matter in the sediments. Thus, if the objective of monitoring is to ascertain the environmental status of the farm, then several transects out from fish cages should be investigated. However, in practice often only one transect has been evaluated in order to keep costs of routine monitoring programs low. This study has also shown the importance of having several reference sites. Although we purposely selected sites to be representative of the normal environmental conditions at the farm, by for example locating them at similar depths generally upstream and downstream of the farming area, at least one reference site at each farm showed some anomalous results. Hideaway Bay reference site R1 had markedly different %TOM, isotope ratios and $\%C_{org}$ and %N compared to other sites. The benthic infauna were also different (Chapter 4). This is probably related to the different sediment particle size composition, but also suggests that other factors were affecting this site. Reference site R1 at Nubeena also showed unexpected results for a number of physical parameters, and the benthic biota data suggested that this site was organically enriched. This site was approximately 600 m from another salmonid farm that had been in operation for a number of years and thus may have been impacted by the farm. Our results clearly emphasise the difficulty in selecting reference sites, and strongly support the need for multiple reference sites to be measured.

In conclusion, our results imply that no one physical/chemical measure of organic enrichment is sufficient for reliable routine monitoring of environmental impacts of salmon farms. Other measures of the effects of organic enrichment, albeit more costly, such as benthic invertebrate composition and abundance, and visual imagery at the surface and within the sediments, are necessary for effective, reliable monitoring.

4. Evaluation Of Benthic Infauna

4.1 Introduction

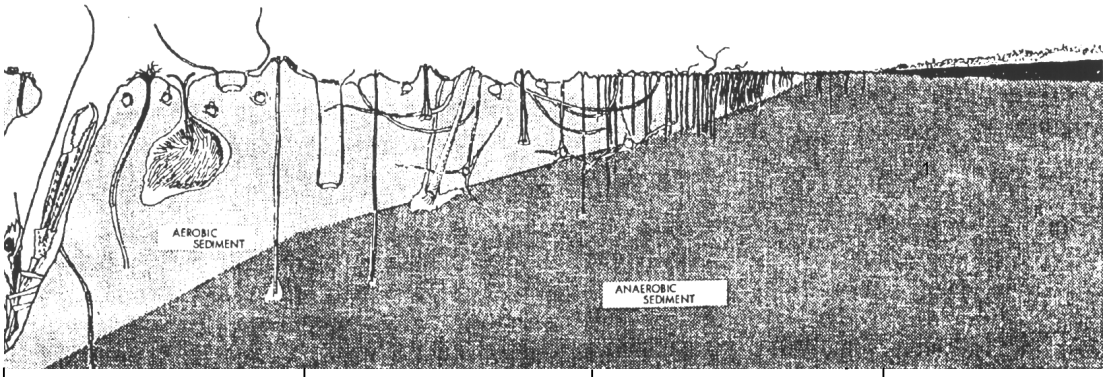
The response of benthic flora and fauna to organic enrichment has been well documented. Pearson and Rosenberg (1978) identified benthic community groups characteristic of four levels of organic enrichment (Fig. 4.1). Effects of organic deposits from fish farms have been shown to exhibit the same community responses (e.g. Brown *et al.*, 1987; Weston, 1990; Hargrave *et al.*, 1997; Karakassis *et al.*, 1998). As the oxygen is depleted, due to the degradation of accumulated fish food and faeces on the bottom, the oxic layer becomes shallower, and the macrofauna, which require oxygen to survive, are driven towards the surface. As the oxygen level in the sediment declines further, many species are eliminated and may be replaced by others more tolerant of a low oxygen environment.

Evaluation of the benthic infauna has been shown in many studies to be the most sensitive indicator of environmental impact resulting from organic enrichment (e.g. Brown *et al.*, 1987; O'Connor *et al.*, 1989; Weston, 1990; Johannessen *et al.*, 1994). Codling *et al.* (1995), in their summary of techniques used for environmental monitoring, determined that evaluation of benthic infauna was a direct and ecologically relevant measure of environmental impact. Also, Weston (1990) observed that the fauna are sensitive at enrichment levels undetectable with gross chemical measures, and that the fauna reflect the integration of effects, which in combination are often more severe than each single event. Consequently, in this study the benthic community structure was assessed for its ability to indicate the effects of organic enrichment from fish farms on the environment. The macrofauna were identified to species level and this was used as the primary assessment against which all further macrofaunal assessment techniques were evaluated.

The benthic infauna were also examined for particular species or faunal groups, which could be used to indicate levels of organic enrichment. Several species have been found to be indicative of organic loadings, most notably the opportunistic polychaete, *Capitella capitata* (e.g. Pearson and Rosenberg, 1978; Brown *et al.*, 1987; Weston, 1990; Lim, 1991; Hargrave *et al.*, 1997). This particular species complex has been identified globally in association with areas of organic enrichment, and has been found at greatly increased densities under fish farms with high organic waste deposits (Pearson and Stanley, 1979; Brown *et al.*, 1987; Weston, 1990; Lim, 1991; Hargrave *et al.*, 1993; Henderson and Ross, 1995). Hargrave *et al.* (1993) actually encountered conditions which were so degraded as to inhibit *Capitella capitata* (the grossly polluted category in Fig. 4.1). Consequently, particular note was taken of the distribution of *Capitella capitata* complex in this study as a potential indicator of impact.

Capitella sp. (MoV 2558) is the current identification code assigned by the Museum of Victoria to the polychaete worm previously identified from South Australia as *Capitella capitata*. There is ongoing discussion as to whether this is a single species or a species

complex; nevertheless, its ecological significance remains the same. For the remainder of the report this species is identified as *Capitella sp.*(MoV2558).



ZONE	NORMAL	TRANSITORY	POLLUTED	GROSSLY POLLUTED
Typical Macrofauna Dominants	Nucula Amphiura Terebellides Rhodine Echinocardium Nephrops	Labidoplax Corbula Goniada Thyasira Pholoe Chaetozone Anaitides Pectinaria Myriochele Ophiodromus	Capitella Scolelepis	No MACROFAUNA Surface covered by bacterial fibre "blanket"

Fig. 4.1. Pattern of community structure change as a result of increasing organic matter loading (from Pearson and Rosenberg, 1978).

The level of taxonomic discrimination required for reliable assessment of impacts of marine farms was also investigated. At present salmon farmers in Tasmania are required by the State Government to periodically monitor the benthic infauna around their farms, with identification to family level. However, assessment of community structure to species level is generally regarded as the most sensitive indicator of benthic condition (Brown *et al.*, 1987; O'Connor *et al.*, 1989; Weston, 1990; Johannessen *et al.*, 1994) and some information may be missed with family level identification. However, several recent studies have indicated that pollution affects the fauna at taxonomic levels higher than species (Warwick 1988a, b; Ferraro and Cole, 1990, 1992; James *et al.*, 1995; Somerfield and Clarke, 1995). Identification to higher taxonomic levels can have considerable benefits, reducing both the time and costs involved in conducting assessments.

Although species level assessments of environmental impact are generally considered to best reflect the true environmental conditions, there are several problems with assessment at this level. Species level identifications are expensive and require a high level of skill and expertise, which may often be unavailable or difficult to access. Bayne *et al.* (1988) suggested that analysis of the data at higher taxonomic level can alleviate both the problem of availability of expertise and that of limited taxonomic information on the fauna. Reliability of the species identifications can be another serious problem with assessment at this level. In areas of the world where the fauna is poorly described, such as in Australia, identification to species level can be very difficult. Ellis (1985) found that unreliable identifications could make significant

differences to the results of analyses. In a trial with a set of six species distributed to nine laboratories claiming taxonomic expertise, less than half got the identifications correct. Such taxonomic inaccuracy can have important biological and functional implications and incorrect identifications may lead to erroneous conclusions about levels of impact. It is also important that the level of identification is compatible with the available ecological information. Organisms should be identified to a level (species, genus, family, etc) which is relevant to the known ecology of that organism (Ellis, 1985). If the ecological significance of the species identification is unknown, then there may be no benefit in identifying the fauna to species level. James *et al.* (1995) looked at the natural variability of the Australian fauna and their results support the idea that identification to species level is useful only if the intrinsic biology of the species is known and relevant, and that identifications are reliable. Several researchers have suggested that if resources are limited, it would be better to direct effort towards greater replication (Warwick, 1988b; Warwick, 1993; James *et al.*, 1995; Bowman and Bailey, 1997). In Tasmania the invertebrate fauna of soft sediments have not been well described, nor is there much information available on the biology of these species.

Many studies have indicated that family level is sufficient to determine the pattern of anthropogenic impact on the marine environment (Warwick, 1988a, b; Ferraro and Cole, 1990, 1992; James *et al.*, 1995; Somerfield and Clarke, 1995). In fact, several studies have suggested that for sublittoral soft sediment benthic macrofauna, little information is lost in multivariate assessment by identification up to phylum level (Ferraro and Cole, 1990; Gray *et al.*, 1990; Warwick 1988c; Warwick *et al.*, 1990). The findings of Ferraro and Cole (1990) indicated that community changes over time as a result of induced stress (due to human or other sources), may be manifested at increasingly higher levels of biological organisation, and therefore the taxonomic level necessary and sufficient to assess change will also increase in a step wise manner equivalent to that proposed by Pearson and Rosenberg (1978). This extends Warwick's hypothesis (1988b) that pollution events affect assemblages at higher taxonomic level than natural disturbances (i.e. above species level) and therefore assessment at a level above species should detect anthropogenic effects. Gray *et al.* (1990) suggested that in some situations high levels of natural variability may actually mask the effects of pollution. Therefore, it is important that any assessment is designed to detect the required level of impact.

Several methods have been employed to analyse the macrofaunal data in the current investigation, including univariate and multivariate techniques. Multivariate assessment of community structure uses the numbers of species and abundance of individuals, in conjunction with the species identities, to distinguish community patterns. Multivariate analyses are thus generally more representative of the community structure. However, other, simpler forms of analysis were also assessed and compared with the multivariate techniques. Evaluation of k-dominance curves has been shown to be a useful method for determining conditions associated with organic enrichment (Lambshead *et al.*, 1983; Beukema, 1988). This technique has the advantage that specific impact levels are clearly associated with particular curve profiles. Many univariate diversity measures have also been applied to assessment of environmental impact, and in particular to assessment of cage aquaculture impacts (Johannessen *et al.*, 1994; Henderson and Ross, 1995; Drake and Arios, 1997; Lu and Wu, 1998). In this study we review the effectiveness of several univariate indices, including species

richness, total abundance and the Shannon Index, as indicators of environmental impact under Tasmanian conditions.

4.1.1 Objectives of the benthic infaunal study

The main objectives of the assessment of benthic macrofauna were:

- To determine if macrofaunal assessment is an effective and reliable technique for evaluating the environmental impact associated with marine finfish aquaculture in Tasmania.
- To determine the level of taxonomic discrimination necessary for reliable detection of farm impact. In particular, to evaluate whether identification to family level is sufficient for identification of environmental effects under Tasmanian conditions.
- To evaluate other, simpler, approaches to using the benthic community to monitor environmental change (i.e. major faunal groups, indicator species).
- To examine univariate and multivariate approaches to analysis of the benthic infaunal assessment data and make recommendations on the most appropriate techniques to use.
- To suggest appropriate categorisations of impact and levels by which these impact categories can be quantified and monitored.

4.2 Methods

4.2.1 Sample collection

At all sites three replicate samples of sediment were collected for benthic analysis either by diver collected cores at the shallower Nubeena sites or by small Van Veen grab in deeper water at Hideaway Bay. Sediment samples were processed on site by rinsing through a 1 mm mesh sieve and preserving the retained material in 4% neutral buffered formalin for a minimum of 48 hours. In the laboratory the samples were rinsed and transferred to 70% ethanol solution. The fauna were identified to the lowest possible taxonomic level and the numbers in each taxa were recorded.

4.2.2 Data analysis

"A priori" it was hypothesised that the benthic community structure along the boundary transects would not be significantly different. Consequently the benthic faunal data for each of the boundary transects were assessed using ANOSIM to determine whether the samples taken at 0 m, 45 m and 60 m points could be combined for each transect. The inshore transects B1 and B2 at Hideaway Bay were treated separately to the outer transects (B3, B4, B5, B6) due to differences in depth and particle size distributions between these transect groups (Chapter 3).

Benthic infaunal data were then analysed using three methods, and the results were compared:

(i) multivariate techniques - cluster analysis, non-metric multidimensional scaling (MDS), analysis of similarity (ANOSIM), identification of representative species by calculation of similarity percentages (SIMPER) and multivariate correlation (RELATE).

(ii) graphical representation (k-dominance curves).

(iii) univariate analyses (counts, diversity indices and analysis of variance (ANOVA)).

The environmental impact of the farm operations was first evaluated at both farm sites by multivariate analysis of species level faunal information. These results were then used to gauge the applicability of simpler assessment techniques: higher taxonomic levels, univariate and graphical analysis and simpler faunal groups and indicator species.

(i) Multivariate techniques

The benthic species community data were compared by multivariate analyses using the PRIMER™ software package (Carr, 1996). A similarity matrix was constructed using the Bray-Curtis similarity index, with the species abundance data being square root transformed before analysis to down weigh the contribution of rare species. Preliminary investigations found that this was adequate to interpret the cluster analyses but still maintain the relative proportional representations of each of the species in the analysis. Patterns in benthic infaunal assemblages at the sample sites were analysed using hierarchial agglomerative clustering and ordination (MDS). The adequacy of the MDS representations were indicated by calculation of stress levels, with stress <0.1 corresponding to a good relationship, and stress >0.2 indicating an unreliable ordination (Clarke and Warwick, 1994). Differences in species abundance between farm cage sites and reference and boundary transect sites were tested using Analysis of Similarity (ANOSIM) techniques (Clarke and Warwick, 1994). The relative contribution of each species to the average similarities of the sites (groups) and average dissimilarities between sites (groups) was calculated and the results expressed as percentages (SIMPER). These results were then used to determine if any particular species were indicative of the patterns identified by cluster and ordination analyses. The level of impact on the benthic fauna was also evaluated from the identification of the key species in each group and by interpreting the ecological relevance of these species groups based on our current understanding of their ecology.

Confidence kernels, which are non-parametric density estimators analogous to a continuous histogram that shows where the data are most concentrated in the sample (SYSTAT graphics manual, 1998), were calculated for the main groups identified by cluster analysis. The 90% level reflects the spread of 90% of the data within the group and is therefore a tighter grouping than the 95% level.

(ii) Graphical techniques

K-dominance plots were introduced by Lambshead *et al.* (1983) as a simple means of graphically representing the distribution of species to facilitate comparisons between samples. This involves plotting the cumulative ranked abundances of species against log species rank. The shape of the curve produced is indicative of the level of environmental disturbance - the higher the curve starts on a plot, the shallower and shorter it is, then the greater the level of disturbance it indicates (Fig. 4.2). If a sample is dominated by only a few species present in large numbers then the associated k-dominance curve will start high and reach the 100% level very quickly. However, if a sample comprises many species, none of which are particularly abundant, as is the case in an undisturbed community, then the curve will start low on the plot and climb gradually.

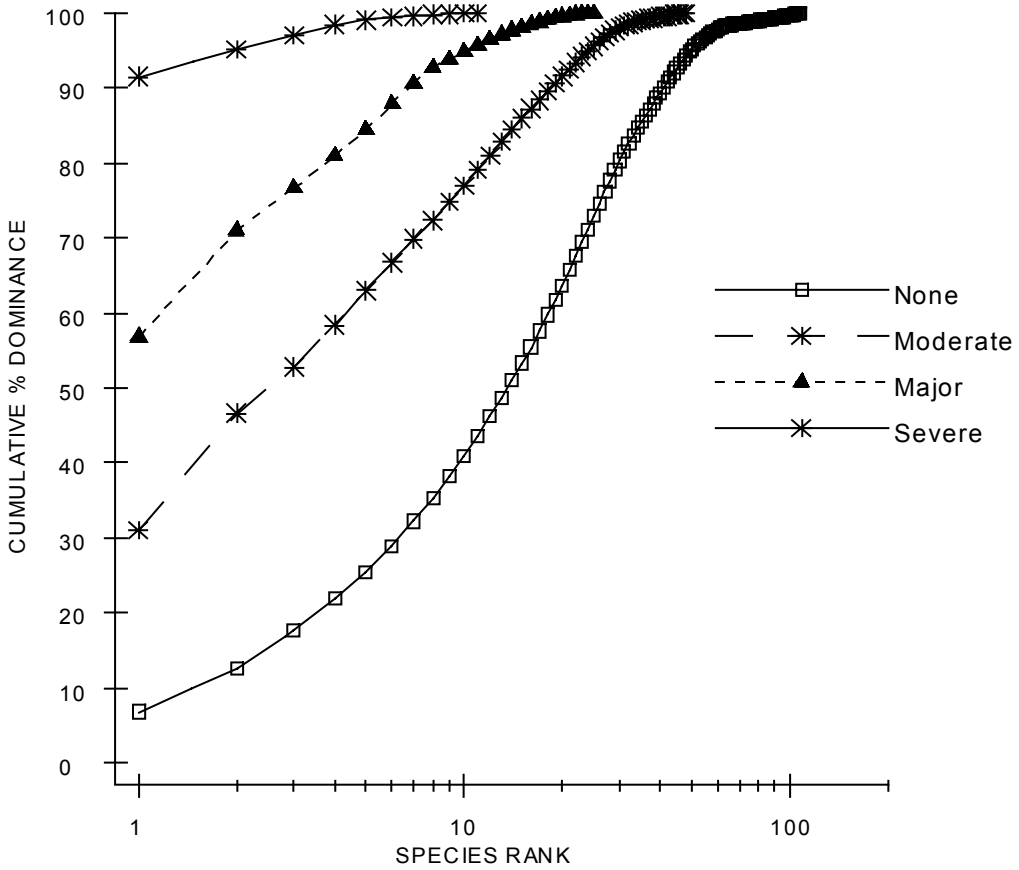


Fig. 4.2. Hypothetical stylised k-dominance curves showing the curve shapes indicative of severe, major, moderate and no disturbance.

(iii) Univariate techniques

High species diversity is generally considered to be indicative of a complex community. A greater variety of species allows for more species interactions, and this in turn suggests community stability and maturity (Brower *et al.*, 1990). High diversity corresponds to many equally or nearly equally abundant species, whereas low diversity suggests very few species or a few highly abundant species.

Indices of diversity which were used in this study have previously been commonly applied in environmental impact assessments and particularly in reference to organic enrichment and aquaculture impacts. They were number of species, total abundance, Shannon index and Inverse Simpson index.

The Shannon index H' indicates the likelihood of picking a particular species from the community, i.e. when an individual is chosen at random from a community with low species diversity it is possible to be more certain of the species identity than would be the case with a highly diverse community.

$$H' = - \Sigma p_I \log p_I$$

where $p_I = n_i/N$, n_i is the number of individuals of species i, and N is the total number of individuals.

Simpson's index considers the number of species (s), the total number of individuals (N) and the proportion of the total that occurs in each species as an indicator of the dominance (Simpson, 1949). The inverse of this index d_s is preferred in situations where the values are very similar and close to 1.

$$d_s = N(N\text{-}1)/ \Sigma n_i(n_i\text{-}1).$$

Univariate data were analysed by Analysis of Variance (ANOVA) and both one-way (where time was not considered a factor) and two-way ANOVA (where site and time interactions were factors) were used. In all cases Tukey's post-hoc test was undertaken for subsequent pairwise comparisons to determine which sites/groups were significantly different.

At Nubeena ANOVA was conducted on both the site results directly and on the "a priori" determined groupings of cages (F1, F2, F3), farm site (F 35), reference sites (R1, R2) and boundary transects (B1, B2). Similarly, at Hideaway Bay analysis was conducted on results from the sites directly, and on the "a priori" determined groupings of reference sites (R1, R2, R3, R4), inshore (B1, B2) and offshore (B3, B4, B5, B6) boundary transects, and the farm cage transect (F 0, F 10, F 35 and F 60).

For evaluation of level of taxonomic discrimination, data were grouped by taxon (species, family, order, class, phylum), and for each taxonomic level, groupings were determined and compared by multivariate analysis. The data were double square-root transformed as this allows low abundance species to take a greater part in the determination of similarities and slightly reduces the dominance of species like *Capitella* on the overall distribution. Groupings obtained for higher taxonomic levels were evaluated in relation to the species level results, both subjectively and by correlation of the similarity matrices underlying the ordinations (RELATE analysis). Data were also compared using univariate indices.

4.3 Results

4.3.1 Comparison of sites along transects

Nubeena

Benthic fauna at 0 m, 45 m, and 60 m sites along the boundary transects were not significantly different (ANOSIM, P>0.05, 0 mo. R = 0.046; 5 mo. R = -0.007); thus transects B1 and B2 were each treated as a single site with 9 replicates at both 0 and 5 months.

Hideaway Bay

Benthic fauna at 0 m, 45 m and 60 m sites along the offshore transects B3, B4, B5 and B6 were not significantly different (ANOSIM, P>0.05) at the 0, 3 and 11 month surveys (0 mo. R = 0.14, 3 mo. R = -0.025, 11 mo. R = 0.031). However, the 45 m site at B5 and B6 was not representative of these transects at 6 months (ANOSIM, 6 mo. R = 0.074, P<0.05). At this time the introduced gastropod *Maoricolpus roseus* was very abundant, consequently only the 0 m and 60 m replicates have been included in subsequent analyses for B5 and B6 at 6 months.

There were also significant differences between the 45 m and 60 m transect positions at 9 months (R = 0.096, P = 0.001). This difference was mainly as a result of changes in the abundances of *Nassarius nigellus, Terebellides stroemii* and *Amphiura elandiformis* at the 45 m and 60 m sites on the B4 transect compared with the other boundary transects. The brittle star *Amphiura elandiformis* was more abundant in other boundary samples than at the B4-45/60m sites whilst conversely, the little dog whelk *Nassarius nigellus* was more abundant at these sites. Both species were found in moderate abundances at the reference locations. *Terebellides stroemii* was absent from the B4-45/60m sites but occurred in relatively high numbers at the reference locations and at most boundary transects.

There were no other differences identified between sites at the offshore transects. Therefore, except where already indicated, sites have been combined into transects in order to simplify data representation.

The 0 m sites of the inshore transects B1 and B2 at Hideaway Bay were located within the farm predator enclosure, and have been within the farmed lease area for many years. Thus B1 and B2 were predicted to have changes in the benthic community along these transects. Analysis of sites along these transects by ANOSIM at each sampling time indicated that the 45 m and 60 m sites had similar community structures and therefore could be combined. However, the fauna at the 0 m sites was significantly different to the 45 and 60 m sites at 0, 3 and 6 months, thus in all analyses the 0 m sites have been treated separately, and the 45 and 60 m sites have been combined and are represented as B*45/60. The differences between the 0 m and remaining sites were as a result of changes in abundances rather than species replacement. The dominant species at these

transects were relatively motile opportunists; the dog whelk, *Nassarius nigellus*, and the bivalve *Mysella donaciformis*.

4.3.2 Assessment of macrobenthic community structure by multivariate analysis

Nubeena

"A priori" groupings of boundary, cage, farm and reference sites were shown to be significantly different by ANOSIM (Table 4.1). Pairwise comparisons showed both the boundary and reference sites to be significantly different from the cage and farm sites.

The most important species at the cage group were predominantly opportunistic (Table 4.2) - the phoxocephalid amphipod *Birubius cartoo,* the dog whelk *Nassarius nigellus*, the bivalve *Mysella donaciformis* and the renowned indicator of organic enrichment *Capitella* sp (MoV2558), which is tolerant of high organic loadings and extremely reduced oxygen levels. Phoxocephalids are a large group of amphipods with a broad range of environmental tolerance. They are mainly benthic burrowers but are relatively mobile and have often been found in areas of organic enrichment (Barnard and Drumming, 1978). However, in contrast the heart urchin *Echinocardium cordatum* was also a key species in the fauna although this species is not usually associated with areas of high organic enrichment. The composition of the farm group fauna was similar to that of the cage fauna. However, neither *Capitella* sp (MoV2558) nor *Nassarius nigellus* were as important, being replaced by the surface deposit feeding polychaete *Polycirrus* sp. (cf *tesselatus*) and the epifaunal cumacean *Cyclaspis caprella*. *Polycirrus* sp. (cf *tesselatus*) is likely to be relatively intolerant of high levels of organic deposition because it is relatively immobile and feeds by spreading its tentacles either across the sediment surface or into the water column. It would therefore be smothered in areas with high levels of sedimentation.

The greatly increased abundance of *Capitella* sp (MoV2558) at the cage sites resulted in this being the most important species in determining the difference between cage sites and both reference and boundary sites.

The species which most clearly distinguished between the farm sites and the boundary/reference groups differed. Between the farm and boundary groups a species of phoronid was the most important discriminator, as this species was not found at the farm sites. The bivalve *Theora fragilis* was also absent from the farm sites; however, the phoxocephalid amphipod *Birubius cartoo* was more abundant. *Pista australis* and *Maoricolpus roseus* occurred at higher densities in the boundary group. The difference between the farm and reference groups could be attributed to the increased abundance of the introduced New Zealand screwshell, *Maoricolpus roseus* at the reference sites.

Table 4.1. One-way ANOSIM of sample site groups based on "a priori" group classification (group 1 - Boundary, group 2 - Cage, group 3 - Farm and group 4 - Reference).

Sample statistic (Global R): 0.585
Significance level of sample statistic: 0.0%

Groups Used	Statistical Value (R)	Significance Level
(1, 2)	0.668	0.001
(1, 3)	0.981	0.029
(1, 4)	-0.112	0.722
(2, 3)	-0.064	0.645
(2, 4)	0.769	< 0.001
(3, 4)	0.867	0.018

Table 4.2. Five most important species in each of the "a priori" defined groups (group 1 - Boundary, group 2 - Cage, group 3 - Farm and group 4 - Reference).

Group 1 – Boundary stations- B1 and B2.
Group Average Similarity – 51.87

Species	Av. Abundance	Ratio	Cumulative %
Pista australis	406.82	5.94	5.84
Polycirrus sp. (cf tesselatus)	138.71	3.15	9.33
Phyllamphicteis (cf foliata)	108.31	4.94	13.91
Nemertea sp	76.54	4.78	17.73
Lumbrinereis sp (MoV322)	92.53	2.99	21.30

Group 2 – Cage stations – F1, F2 and F3
Group Average Similarity – 38.34

Species	Av. Abundance	Ratio	Cumulative %
Birubius cartoo	351.54	3.62	12.73
Capitella sp (MoV2558)	199922.57	0.84	24.51
Echinocardium cordatum	127.64	2.73	33.89
Mysella donaciformis	169.49	1.57	42.72
Nassarius nigellus	221.80	2.79	50.99

Group 3 – Farm stations – F.35 m
Group Average Similarity – 45.51

Species	Av. Abundance	Ratio	Cumulative %
Birubius cartoo	251.10	2.62	11.81
Echinocardium cordatum	131.83	8.63	22.97
Cyclaspis caprella	75.33	6.40	33.04
Mysella donaciformis	69.05	6.05	42.70
Polycirrus sp. (cf tesselatus)	75.33	4.64	52.19

Group 4 – Reference stations – R1 and R2
Group Average Similarity – 39.11

Species	Av. Abundance	Ratio	Cumulative %
Polycirrus sp. (cf tesselatus)	459.99	3.09	11.11
Maoricolpus roseus	631.38	2.47	20.80
Pista australis	252.83	2.60	28.21
Nephtys australiensis	80.04	3.04	35.46
Nemertea sp	63.56	3.26	41.48

The benthic fauna were clearly separated into two main groups by Cluster Analysis, with a similarity level of less than 20% (Fig. 4.3). Group 1 consisted of all reference and boundary sites and group 2 of all farm and cage sites. The next dichotomy, at a similarity level of 25%, separated the farm sites into group 2a which comprised most of the sites next to farm cages at the 5 and 10 month surveys, and group 2b which contained the sites next to cages at the initial sampling, the site 35 m from the cages at all sample times, and the cage site F3 at 5 months.

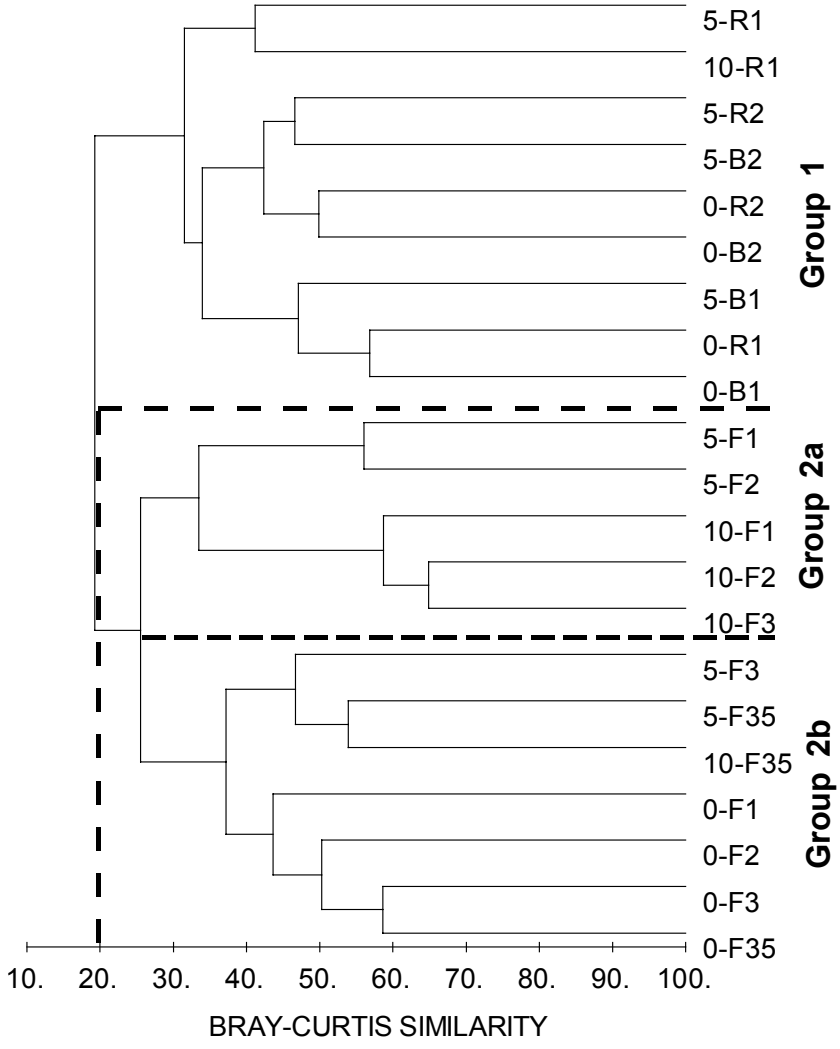


Fig. 4.3. Dendogram plot showing the results of cluster analysis of species from all sites at Nubeena. Numbers prefixed to sites are sampling times in months.

SIMPER analysis of the multivariate groupings (Table 4.3) did not indicate any species which could be described as characteristic of groups 1 or 2b. The fauna of these groups was not dominated by any particular species. Of the five most important species in group 1, two were surface feeding polychaetes (*Polycirrus* sp.(cf *tesselatus*) and *Pista australis*). The introduced New Zealand screwshell (*Maoricolpus roseus*) was the most abundant species in group 1. Two crustacean species were prevalent at group 2b, the phoxocephalid amphipod, *Birubius cartoo,* and the cumacean, *Dimorphostylis cottoni* which together accounted for 18% of the within group similarity. In group 2a the capitellid polychaete *Capitella sp.* (MoV 2558) alone comprised 27% of the within

group similarity, and was responsible for much of the differentiation between groups. *Capitella sp.* (MoV 2558) was the species which most clearly indicated the difference between group 1 and group 2 (a and b). The average abundances of *Capitella sp.* (MoV 2558) were 35,770 m^{-2} for the group 2a sites, and 6 and 94 m^{-2}, respectively, for groups 1 and 2b (Table 4.8). On the basis of the known ecology of this particular species, the cluster analysis groupings could be classified as: Group 1 - no impact, Group 2a - major impact, and Group 2b - moderate impact.

Table 4.3. Five most important species in each of the multivariate impact groups identified in Fig. 4.3 at Nubeena (group 1 – no impact, group 2a –moderate impact, group 2b – major impact).

Group 1 - All boundary and reference sites R1, B1, R2, and B2.
Group Average Similarity – 44.82

Species	Av. Abundance	Ratio	Cumulative %
Polycirrus sp (cf tesselatus)	317.20	2.33	6.98
Pista australis	321.27	3.29	13.45
Maoricolpus roseus	487.34	2.31	19.66
Nemertea spp	69.33	3.76	24.51
Nephtys australiensis	65.11	2.06	29.21

Group 2a - Stations 5-F1, 5-F2, 10-F1, 10-F 2 and 10-F3
Group Average Similarity – 45.82

Species	Av. Abundance	Ratio	Cumulative %
Capitella capitata complex	35770.23	4.60	27.06
Birubius cartoo	271.19	2.98	37.91
Echinocardium cordatum	112.99	2.45	45.61
Nassarius nigellus	79.10	3.04	52.36
Mysella donaciformis	124.29	1.07	58.90

Group 2b - Stations 0-F1, 0-F2, 0-F3, 0-F35m, 5-F3, 5-F35m and 10-F35m
Group Average Similarity – 45.11

Species	Av. Abundance	Ratio	Cumulative %
Birubius cartoo	365.89	3.39	11.23
Echinocardium cordatum	139.90	4.04	20.56
Mysella donaciformis	158.73	4.53	29.14
Nassarius nigellus	295.94	3.80	37.18
Dimorphostylis cottoni	137.21	5.52	44.21

The two-dimensional ordination plot of all sites at Nubeena (Fig. 4.4) shows a progression across the plot, in relation to both time and impact, from the top left hand side (background conditions at reference and boundary sites) to the bottom right (sites next to cages after 5 and 10 months with high levels of organic enrichment).

There were no overlaps of the cluster groupings with 90% confidence kernels (Fig. 4.4).

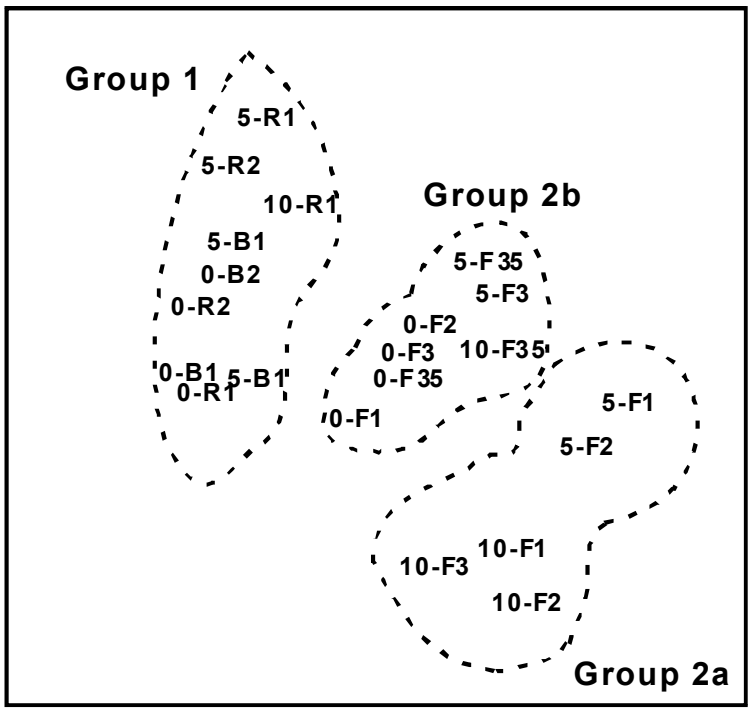


Fig. 4.4. Ordination (MDS) plot of all sites at Nubeena, with 90% confidence kernel ellipses for the cluster groups identified at Nubeena. Stress level = 0.12. Numbers prefixed to sampling sites are sampling times in months.

Hideaway Bay

All of the "a priori" defined groups at Hideaway Bay were significantly different except the farm and reference groups (Table 4.4). SIMPER analysis of these groupings indicated that the opportunist polychaete *Capitella sp*. (MoV 2558) was the most significant species in classifying the cage group (Table 4.5). This species accounted for more than 35% of the within group similarity. The scavenging nassarid, *Nassarius nigellus* and the introduced bivalve *Corbula gibba* accounted for a further 32% of the within group similarity, with these three species being responsible for 67% of the group similarity. The farm group was less strongly characterised by any particular species and *Capitella sp*. (MoV 2558) was not a significant discriminator at this site, although it was present at low levels.

Nassarius nigellus was present at all of the Hideaway Bay groups but was only a significant distinguishing species in relation to the cage group. Echinoderms were only significant in the non-cage groups; *Echinocardium cordatum* at the inshore sites and *Amphiura elandiformis* at the offshore, reference and farm sites.

Table 4.4. One-way ANOSIM of sample station groups based on "a priori" group classification (group 1 – Boundary (inshore), group 2 – Boundary (offshore), group 3 - Cage, group 4 – Farm, group 5 - Reference).

Sample statistic (Global R): 0.425
Significance level of sample statistic: 0.0%

Groups Used	Statistical Value (R)	Significance Level
(1, 2)	0.523	<0.001
(1, 3)	0.769	<0.001
(1, 4)	0.154	0.046
(1, 5)	0.491	<0.001
(2, 3)	0.935	<0.001
(2, 4)	0.599	<0.001
(2, 5)	0.178	<0.001
(3, 4)	0.728	0.001
(3, 5)	0.740	<0.001
(4, 5)	0.155	0.086

Table 4.5. Five most important species in each of the "a priori" defined groups (group 1 - Inshore Boundary, group 2 - Offshore Boundary, group 3 - Cage, group 4 - Farm and group 5 - Reference).

Group 1 – Inshore Boundary stations- B1, B2.
Group Average Similarity – 36.84

Species	Av. Abundance	Ratio	Cumulative %
Nassarius nigellus	653.48	2.92	16.84
Mysella donaciformis	171.37	1.08	26.73
Ecinocardium cordatum	34.84	1.25	33.54
Paraprionospio coora	61.21	0.91	38.14
Lumbrinereis sp. (MoV322)	19.77	0.88	42.54

Group 2 – Offshore Boundary stations- B3, B4, B5, B6.
Group Average Similarity – 62.83

Species	Av. Abundance	Ratio	Cumulative %
Nassarius nigellus	224.47	4.76	8.30
Amphiura elandiformis	147.64	7.00	16.19
Nemertea sp.	44.75	5.57	21.80
Mediomastus australiensis	36.63	5.56	27.11
Thyasira adelaideana	22.38	7.27	31.84

Group 3 – Cage stations – F.0m, F.10m
Group Average Similarity – 45.84

Species	Av. Abundance	Ratio	Cumulative %
Capitella sp. (MoV 2558)	1468.52	1.96	35.59
Nassarius nigellus	197.06	7.46	57.21
Corbula gibba	10.22	1.60	67.82
Malacoceros tripartitus	8.02	0.72	73.77
Nematoda sp.	51.75	0.73	79.07

Group 4 – Farm stations – F.35m, F.60m
Group Average Similarity – 54.94

Species	Av. Abundance	Ratio	Cumulative %
Nassarius nigellus	593.66	3.30	10.05
Nemertea sp.	127.46	3.89	17.36
Amphiura elandiformis	159.24	2.90	24.13
Mediomastus australiensis	72.48	4.44	30.59
Theora fragilis	86.27	2.15	36.44

Table 4.5 continued

Group 5 – Reference stations – R1, R2, R3 and R4.
Group Average Similarity – 39.19

Species	Av. Abundance	Ratio	Cumulative %
Amphiura elandiformis	117.76	1.10	11.07
Nassarius nigellus	55.05	1.59	20.65
Nucula pusilla	26.77	1.39	27.77
Nemertea sp.	21.86	1.33	34.63
Mediomastus australiensis	19.50	1.38	41.24

Cluster analysis of Hideaway Bay infauna (Fig. 4.5) showed that the primary dichotomy, at a similarity level of approximately 18%, distinguished all F 0 m sites and most F 10 m sites (Group 2) from all remaining boundary and reference sites (Group 1). High abundances of *Capitella sp*. (MoV 2558) (~ 1600 m^{-2}) and low abundances of the brittle star *Amphiura elandiformis* characterised the group 2 sites (Table 4.10). Within group 2 *Capitella sp*. (MoV 2558) accounted for 30% of the overall similarity.

Group 1 could be divided into two further groups at a similarity level of ~ 23% (Fig. 4.5), and this division broadly separated the inshore sites within the predator fence from the other boundary and reference transects. Group 1 was most clearly characterised by the dog whelk *Nassarius nigellus* and the bivalve *Mysella donaciformis*, and these two species accounted for approximately 32% of the overall similarity within group 1. Group 1 could be distinguished from Group 2 by the abundance of *Capitella sp*. (MoV 2558) (Table 4.6). This species was present in much greater numbers at the group 2 sites (~ 1,359 m^{-2}) than at the group 1 sites (~ 16 m^{-2}). In contrast *Mysella donaciformis* was more abundant at group 1 than at any of the group 2 sites. The subgroups 1a and 1b could be individually distinguished from group 2 as a result of the abundances of *Capitella sp*. (MoV 2558), 10 m^{-2}at group 1a and 22 m^{-2} at group 1b. *Nassarius nigellus* was more abundant at the group 1a sites than at either group 1b or group 2. *Mediomastus australiensis* was a characteristic species in group 1b but abundances of this species and of *Amphiura elandiformis* were much lower at the group 2 sites (results not shown).

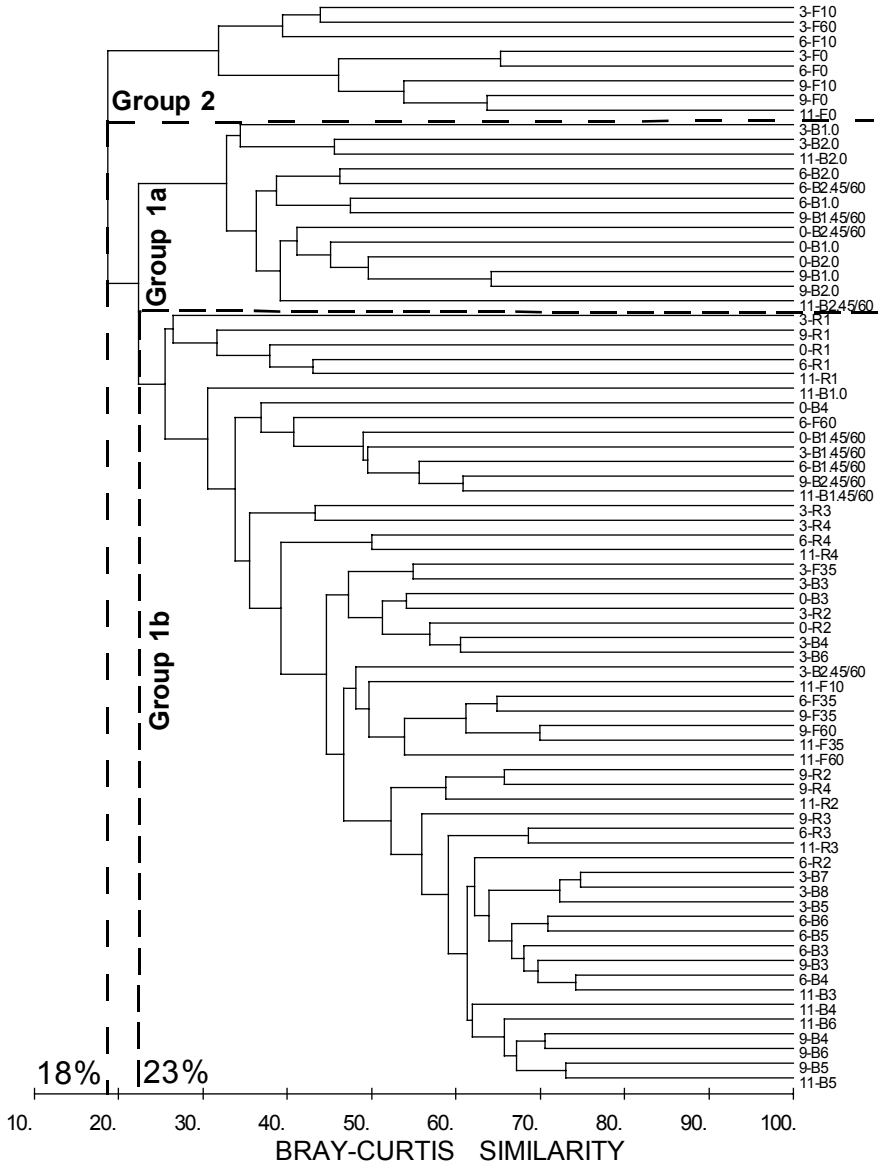


Fig. 4.5. Dendogram showing the results of cluster analysis of sites at Hideaway Bay. Numbers prefixed to sites are sampling times in months.

Table 4.6. Five most important species in each of the multivariate impact groups identified in Fig. 4.5 at Hideaway Bay

Group 1a -.
Group Average Similarity – 37.93

Species	Av. Abundance	Ratio	Cumulative %
Nassarius nigellus	794.39	5.16	20.61
Nucula pusilla	250.81	1.90	36.83
Echinocardium cordatum	35.10	0.96	42.91
Lumbrinereis sp (MoV322)	22.26	0.95	48.35
Euphilomedes sp. (MoV18)	47.94	0.77	52.98

Group 1b - Stations
Group Average Similarity – 42.56

Species	Av. Abundance	Ratio	Cumulative %
Nassarius nigellus	258.55	2.24	10.76
Amphiura elandiformis	114.49	1.35	19.16
Mediomastus australiensis	38.46	1.80	25.81
Nemertea sp.	42.33	1.63	32.16
Aphelochaeta sp. (MoV752)	20.94	1.25	36.89

Group 2 - Stations
Group Average Similarity – 36.24

Species	Av. Abundance	Ratio	Cumulative %
Capitella capitata complex	1358.71	1.08	29.92
Nassarius nigellus.	222.74	2.54	49.99
Corbula gibba	14.09	1.25	59.52
Nematoda sp.	68.65	1.30	68.84
Nemertea sp	41.74	0.91	75.93

Between Group Dissimilarity
Group Average Dissimilarity – 72.16

	Group 1b	Group 1a		Cumul. %
Species	Av. Abundance	Av. Abundance	Ratio	Dissimilarity
Mysella donaciformis	15.39	250.81	1.86	4.02
Amphiura elandiformis	114.49	13.70	1.48	6.97
Nassarius nigellus	258.55	794.39	1.15	9.25
Mediomastus australiensis	38.46	21.40	1.48	11.39
Nemertea sp.	42.33	14.55	1.37	13.52

Group Average Dissimilarity – 78.70

	Group 2	Group 1a		Cumul. %
Species	Av. Abundance	Av. Abundance	Ratio	Dissimilarity
Capitella capitata complex	1358.71	10.27	1.21	6.79
Mysella donaciformis	2.02	250.81	1.92	11.78
Nassarius nigellus	222.74	794.39	1.25	14.54
Echinocardium cordatum	0.00	35.10	1.37	17.29
Nematoda sp.	68.65	5.14	1.22	19.83

Group Average Dissimilarity – 71.77

	Group 2	Group 1b		Cumul. %
Species	Av. Abundance	Av. Abundance	Ratio	Dissimilarity
Capitella capitata complex	1358.71	22.40	1.24	6.60
Amphiura elandiformis	56.15	114.49	1.18	9.80
Nematoda sp.	68.65	22.95	1.15	12.29
Mediomastus australiensis	27.12	38.46	1.21	14.60
Theora fragilis	19.54	30.69	1.18	16.84

Group 2, shown on the far left of the MDS plot (Fig. 4.6), contained all the sites adjacent to the cage of salmon (F 0 m). It also contained several of the sites 10 m from the cage boundary. The faunal community associated with this group was representative of organically enriched conditions. Groups 1a and 1b were equidistant from Group 2 on the right side of the plot, indicating similar effects of organic enrichment at both these groups.

The 90% confidence kernels around the site groupings (Fig. 4.6) showed no overlap for groups 1a or 1b, although a few sites lie outside the kernels. However, there is an overlap in the confidence kernels for groups 1b and 2. This is largely as a result of the F 60 m site clustering in group 2. The faunal community at F 60 m was very diverse and included species which would not normally be expected to occur in a very fine substrate. A rock outcrop adjacent to the site most likely influenced this community structure. The positions of the F 10 m sites also suggest that the impact at this site increased over time.

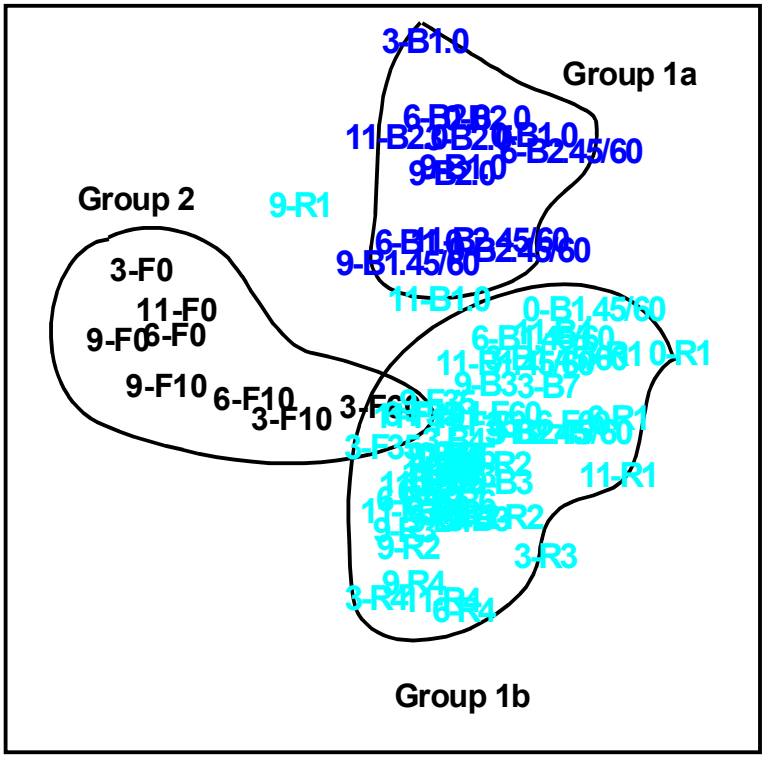


Fig. 4.6. Ordination (MDS) plot of all sites at Hideaway bay, with 90% confidence kernel ellipses for the cluster groups. Stress level = 0.18. Numbers prefixed to sampling sites are sampling times in months.

Based on the known ecology of the dominant species occurring at the different sites at Hideaway Bay and the environmental conditions described in Chapter 3, the cluster analysis groupings were classified as: Group 2 - major impact, and Groups 1a and 1b - minor impact. All sites at Hideaway Bay, including the reference sites, had much higher background levels of organic enrichment than at Nubeena (Chapter 3), thus the fauna appears to be already predisposed to enriched conditions.

4.3.3 K-dominance curves

Although the full dataset was analysed, only the results of representative sites/groups are shown in order to simplify the representation of many of the following analyses and to facilitate interpretation.

Nubeena

The k-dominance curves for all sites at 0 months were similar (Fig. 4.7), and fitted the profile of an undisturbed community. The first ranked species in all cases accounted for less than 30% of the cumulative dominance.

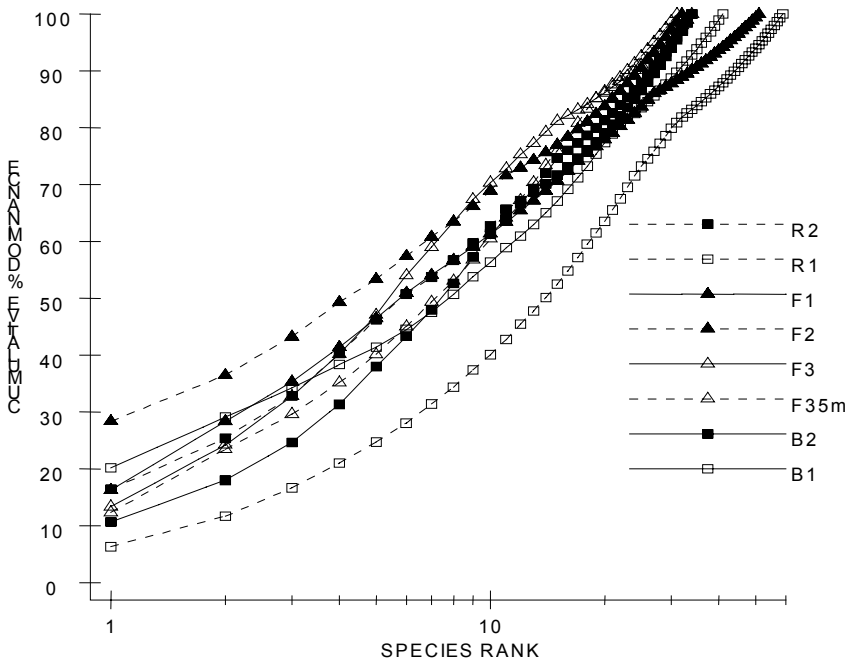


Fig. 4.7. k-dominance curves of individual sites at Nubeena at the initial 0 month sampling. Plots for B1 and B2 are for the 35 m sites only.

At the 5 month sampling, curves for F1, F2, F35 and R1 sites no longer displayed the undisturbed community curve (Fig. 4.8). The first ranked species at the F2 site comprised approximately 60% of the cumulative dominance at that site and the curve was consistent with a community structure with major impact. At both the R1 and F 35 m sites the cumulative dominance of the first ranked species was also increased (approximately 52% and 43%, respectively) and these curves indicated moderately impacted conditions, as did F1.

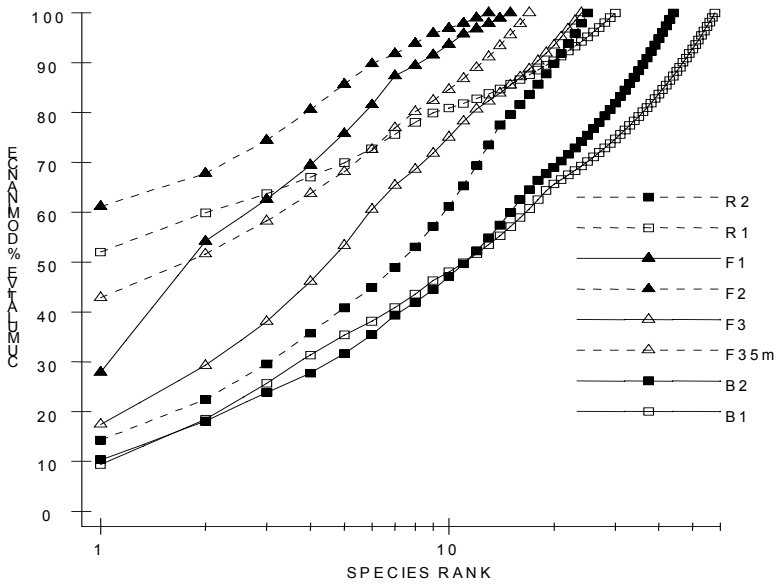


Fig. 4.8. k-dominance curves of the individual sites at Nubeena during the sampling at 5 months. Plots for B1 and B2 are for the 35m sites only.

Plots for all of the cage sites F1, F2 and F3 after 10 months were characteristic of major - severely impacted conditions (Fig. 4.9), and the first ranked species dominance was greater than 60% at all three sites. The curve for the R1 site still indicated moderately impacted conditions whilst the F35 site displayed a curve which reflected unimpacted conditions.

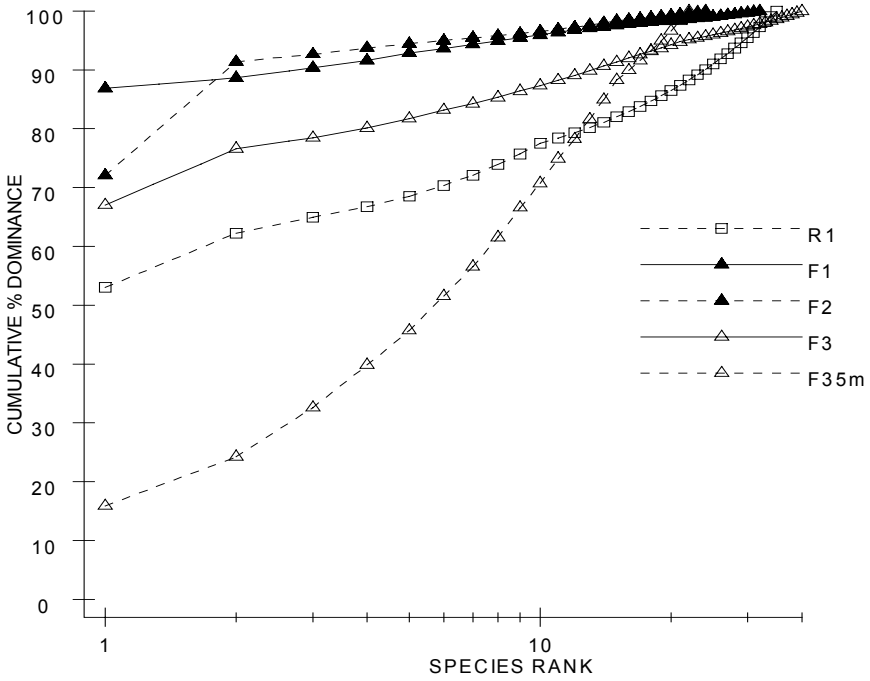


Fig. 4.9. k-dominance curves of individual sites at Nubeena at the 10 month sampling.

The k-dominance curves of the sites identified by multivariate analysis to be major-severely impacted (Group 2a) also indicated major-severe conditions (Fig. 4.10). These

curves all started high (above 60%) on the cumulative dominance axis. The group 2b sites, identified by multivariate analysis as showing a moderate impact, had k-dominance curves indicative of undisturbed conditions. The k-dominance curves for the group 1 sites (unimpacted by multivariate techniques) also generally represented undisturbed conditions with the exception of the curve for site R1 at 10 months (…■…) which indicated a moderate disturbance.

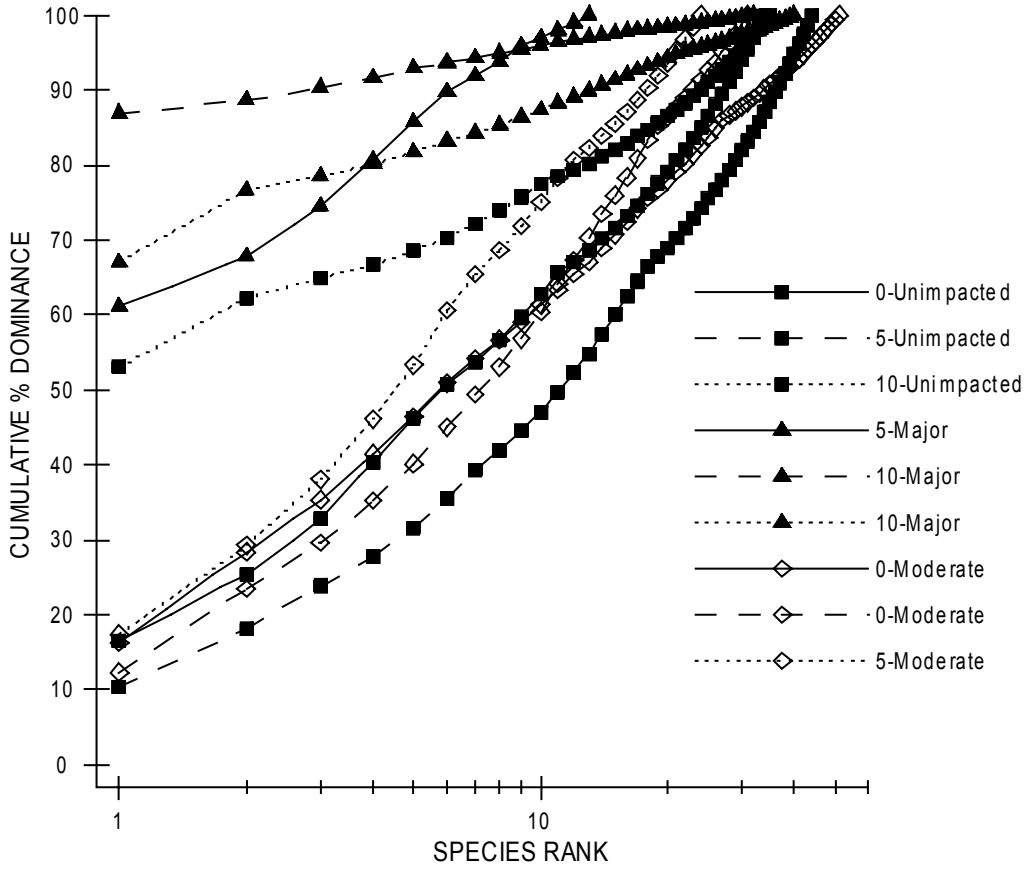


Fig. 4.10. k-dominance curves of sites at Nubeena which have been identified by multivariate benthic community analysis as unimpacted (Group 1), moderate impact (Group 2b) and major-severely impacted (Group 2a). Numbers prefixed to groups are sampling times in months.

The plots in Fig. 4.11 show the cumulative effects of organic inputs at several sites over time. At the start of sampling the k-dominance curve for F1 represented unimpacted conditions, after 5 months it indicated a moderate impact and after 10 months a major/severe impact. However, B2 showed an unimpacted curve profile for the duration of the study. The reference location R1 indicated a moderate impact at 5 months and maintained this curve structure at 10 months.

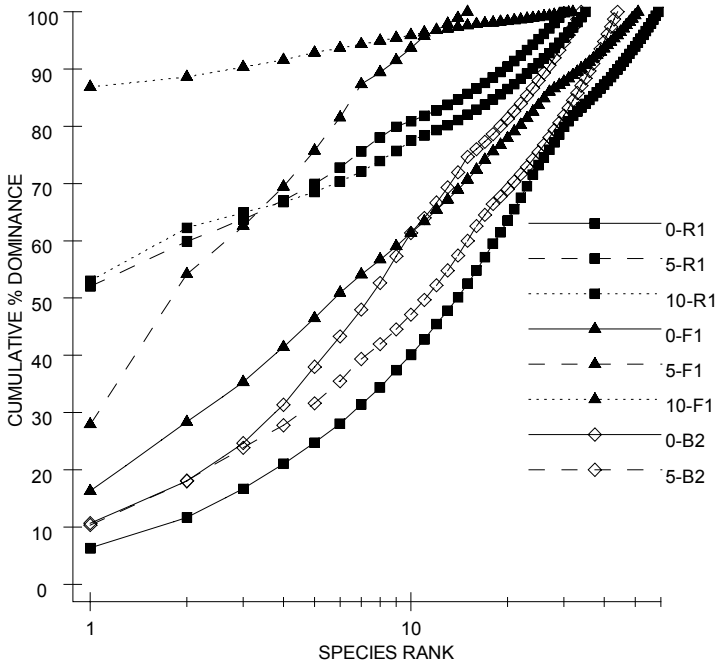


Fig. 4.11. k-dominance curves showing change over time at sites representative of control (R1), cage (F1) and boundary/35 m (B2) conditions at Nubeena.

Hideaway Bay

At 3 months all Hideaway Bay sites except F 0 m and R4 displayed curves indicative of unimpacted conditions (Fig. 4.12). The F 0 m site showed a moderate impact and R4 a minor/moderate impact.

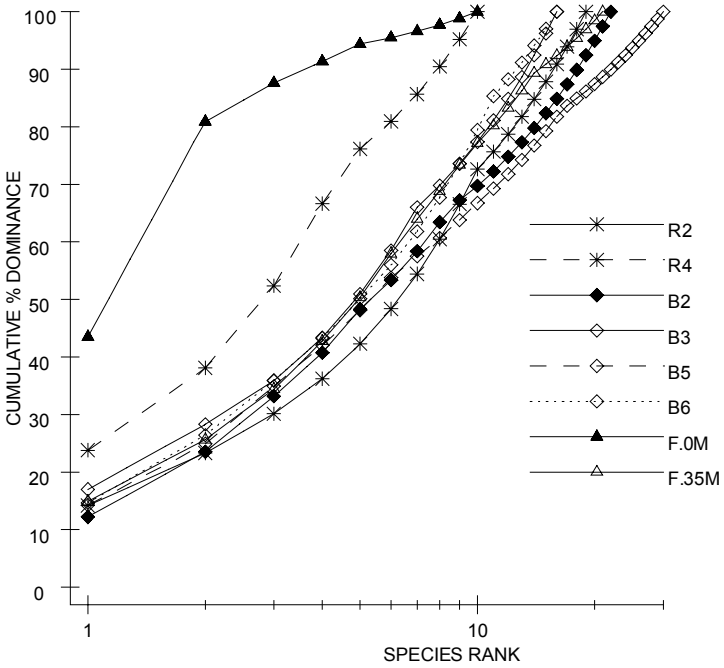


Fig. 4.12. k-dominance curves of representative farm, reference and boundary (35 m) sites at Hideaway Bay at the 3 month survey.

At the 6 monthly sampling the plots suggested a greater spread of conditions (Fig. 4.13). Sites R2, B3, B5 and B6 still indicated unimpacted conditions, whilst at sites F 0 m, B2, F 35 m and R4 the plots suggest that conditions were moderately impacted. No sites displayed major/severely impacted conditions.

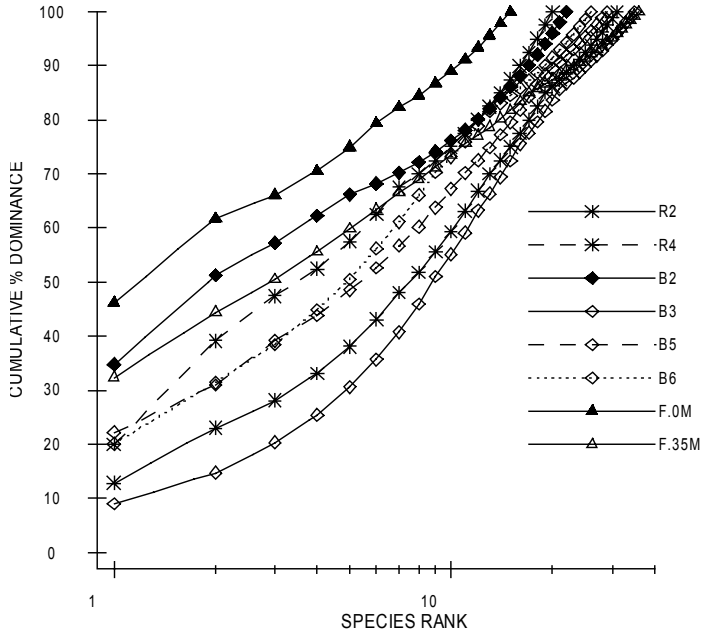


Fig. 4.13. k-dominance curves showing the spatial variability of representative farm, reference and boundary (45m) sites at Hideaway Bay for the 6 month survey.

After 9 months the F 0 m curve showed a severely impacted curve profile (Fig. 4.14). The remaining sites ranged from unimpacted to moderately impacted. B2 and B3 most clearly represented unimpacted conditions whilst the R4 site was moderately impacted.

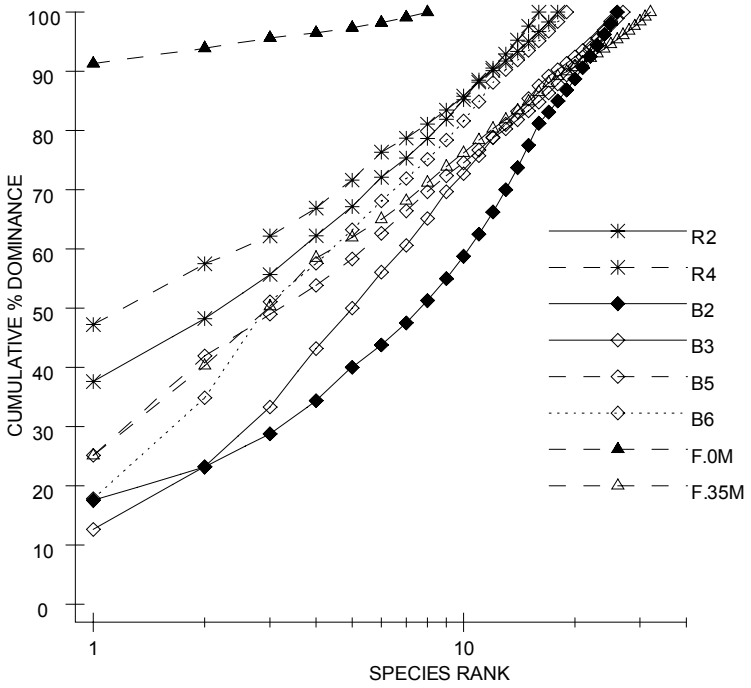


Fig. 4.14. k-dominance curves of representative farm, reference and boundary (35 m) sites at Hideaway Bay for the 9 month survey.

At 11 months the F 0 m curve was still clearly identifiable as impacted, whilst all the remaining sites were unimpacted (Fig. 4.15).

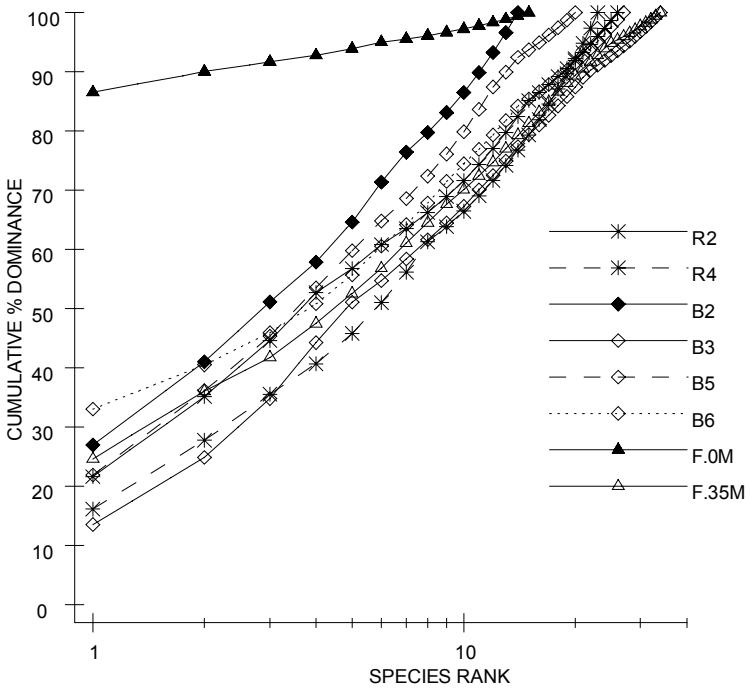


Fig. 4.15. k-dominance curves of representative farm, reference and boundary (35m) sites at Hideaway Bay for the 11 month survey.

The k-dominance curves for sites representative of the differing levels of impact identified by multivariate analysis showed that F 0 m at 9 months (…▲..) was the only

site indicative of severely impacted conditions (Fig. 4.16). The cumulative dominance of the first ranked species for this site and time was greater than 90%. The two other sites in cluster group 2 (major impact) showed profiles consistent with a moderate impact. Only site 6-F 60 m (-*-, unimpacted) showed a fully undisturbed curve profile, all the other multivariate unimpacted (group 1) sites reflected a minor or moderate impact.

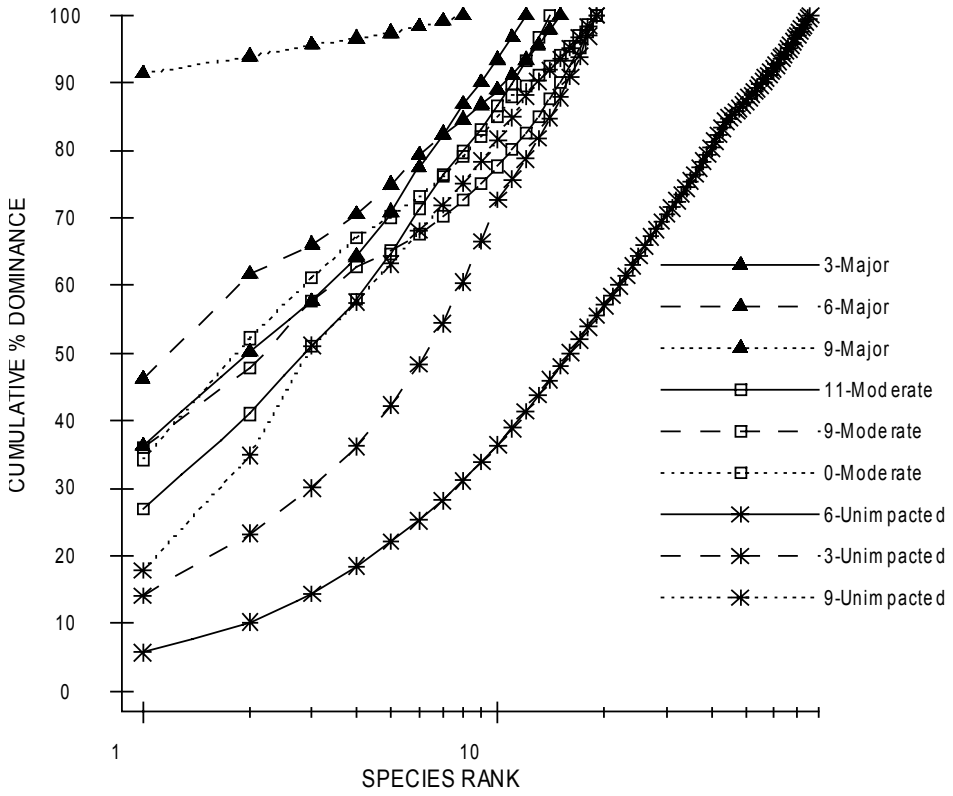


Fig. 4.16. k-dominance curves of sites at Hideaway Bay which have been identified by multivariate benthic community analysis as unimpacted (Group 1b), moderately impacted (1a) and highly impacted (Group 2). Numbers prefixed to groups are sampling times in months.

Over the sampling period the curves for the F 0 m site (Fig. 4.17) indicated a progressively increasing impact. At the 3 and 6 month surveys the curves showed a moderate impact, but this changed to a major/severe impact on the last two sampling occasions. Conditions at the F 35 m station also suggested a temporal progression from unimpacted at 3 months to a minor/moderate impact at all subsequent surveys.

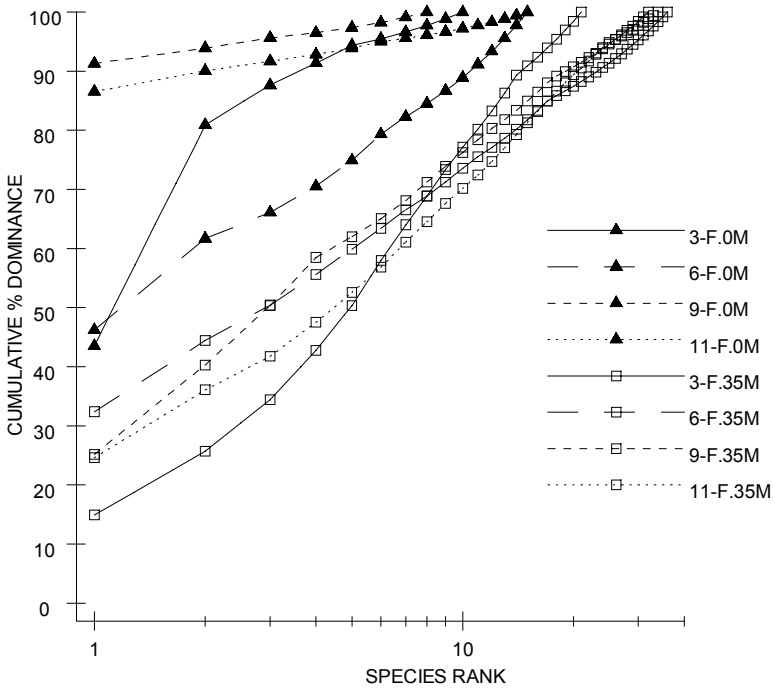


Fig. 4.17. k-dominance curves showing the temporal variability of F 0 m and F 35 m sites at Hideaway Bay.

4.3.4 Major faunal groups and diversity indices

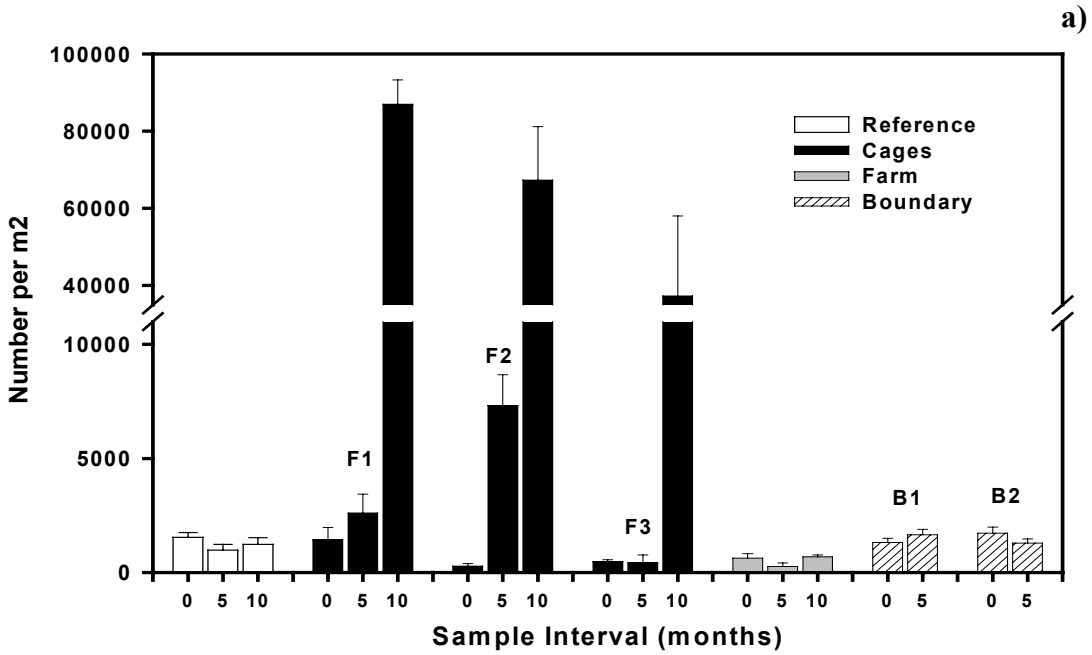
Nubeena

At Nubeena 16,757 individuals from 309 species were identified in this study. Abundance of individuals in four major faunal groups and of the organic enrichment indicator species *Capitella* sp. (MoV 2558) were examined for changes which could be related to levels of organic enrichment (Table 4.7).

Table 4.7. Mean, minimum and maximum numbers of individuals m^{-2} within the major faunal groups for the main site groupings at Nubeena.

		Annelida	Crustacea	Mollusca	Echinodermata	*Capitella* sp.
REFERENCE	**mean**	**1 254**	**659**	**652**	**68**	**8**
	min	169	57	0	0	0
	max	2 090	2 542	2 316	508	113
BOUNDARY	**mean**	**1 482**	**648**	**558**	**88**	**1**
	min	395	0	0	0	0
	max	2 994	1 926	2 599	395	57
CAGE	**mean**	**22 680**	**1 536**	**448**	**138**	**19 923**
	min	57	169	0	0	0
	max	93 503	3 842	1 977	395	92 599
FARM	**mean**	**521**	**734**	**257**	**132**	**69**
	min	57	0	0	0	0
	max	1 017	1 299	678	282	282
NON-CAGE	**mean**	**1 419**	**651**	**584**	**82**	**3**
(Ref & Boundary)	min	169	0	0	0	0
	max	2 994	2 542	2 599	508	113

Densities of annelids at Nubeena were markedly higher at sites next to cages than at any other sites (mean 22,680 compared with 1,419 at reference and boundary sites) (Table 4.7, Fig. 4.18). The average number m^{-2} at the cage sites during the final 10 month sampling (major impact) was 63,867, and *Capitella* sp. (MoV2558) was clearly the dominant species (Table 4.7, Fig. 4.18). Two-way ANOVA of the annelid abundance data for the four site groupings identified in Fig. 4.18 over time showed a highly significant interaction between site and time ($N = 75$, $df = 6$, $F = 20.487$, $p<0.001$ for all sites at 0 and 5 months; $N = 51$, $df = 8$, $F = 13.611$, $p<0.001$ for F1, F2, F3, F35 and R1 sites at 0, 5 and 10 months). Pairwise comparisons indicated a significant difference between the F1 and F2 sites and all other sites at 10 months. Two-way ANOVA of *Capitella* sp. (MoV2558) abundance data also indicated a highly significant interaction between site and time ($N = 51$, $df = 8$, $F = 6.118$, $p<0.001$ for Cage, Farm and Reference groups at 0, 5 and 10 months; $N = 75$, $df = 6$, $F = 51.471$, $p<0.001$ for all sites at 0 and 5 months) and pairwise comparisons identified that the cage group was significantly different to all others. The abundance of annelids was strongly correlated with the abundance of *Capitella* sp. (MoV2558), ($r = 0.974$, $N = 90$, $p=0.01$).



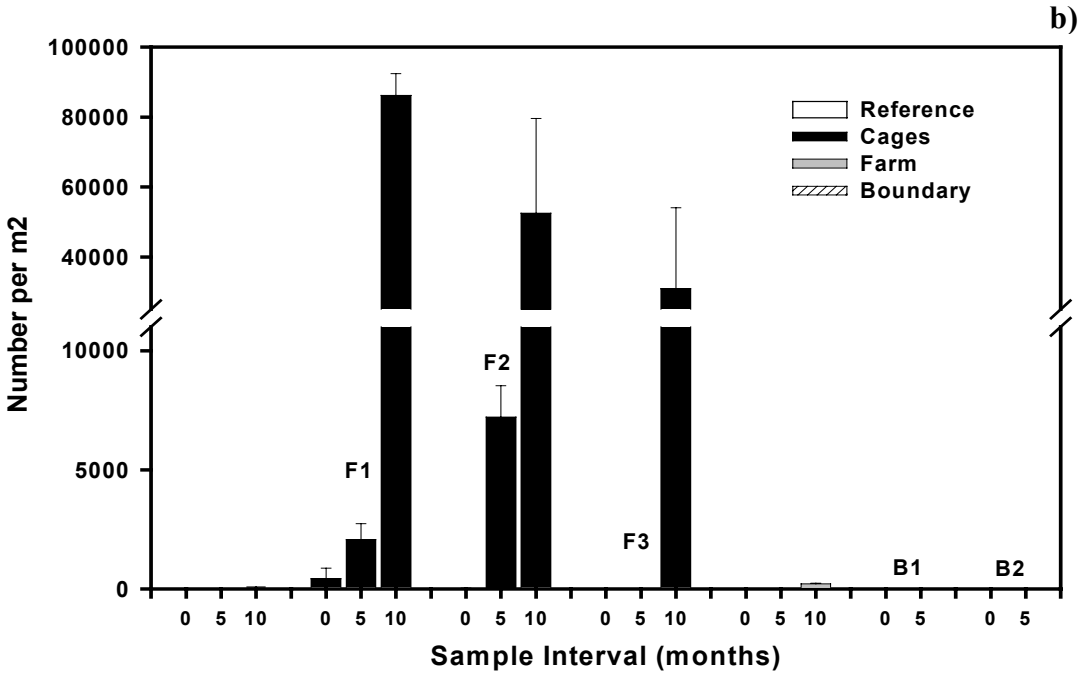


Fig. 4.18. Number m^{-2} of a) annelids and b) *Capitella* sp.(MoV2558) at Nubeena sites.

Crustacean densities were highest next to the cages and were similar between reference, boundary and farm (F 35) sites (Fig. 4.19, Table 4.7). Two-way ANOVA indicated significant differences over time (N = 75, df = 1, F = 12.112, p = 0.001 for all sites at 0 and 5 months; N = 51, df = 2, F = 7.011, p = 0.003 for Cage, Farm and Reference groups at 0, 5 and 10 months) and between sites (N = 75, df = 6, F =15.833, $p<0.001$ for all sites at 0 and 5 months; N = 51, df = 4, F = 11.408, $p<0.001$ for Cage, Farm and Reference groups at 0, 5 and 10 months). However, the interaction of site and time was not significant. Pairwise comparisons indicated that for the most part site F1 was significantly different from the other sites.

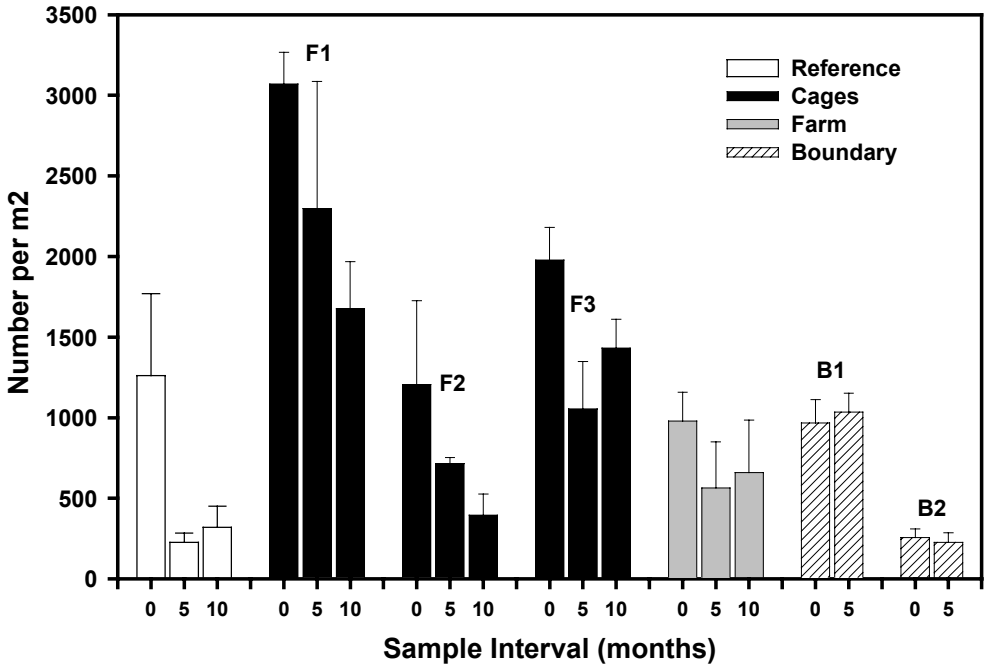


Fig. 4.19. Number per m^2 of crustacea at all sites at Nubeena.

Echinoderm abundances were more variable across sites. Overall, abundance appeared higher at farm and cage sites than at reference and boundary sites (Table 4.7, Fig. 4.20) but the variability between replicates was high, and these differences were not significant.

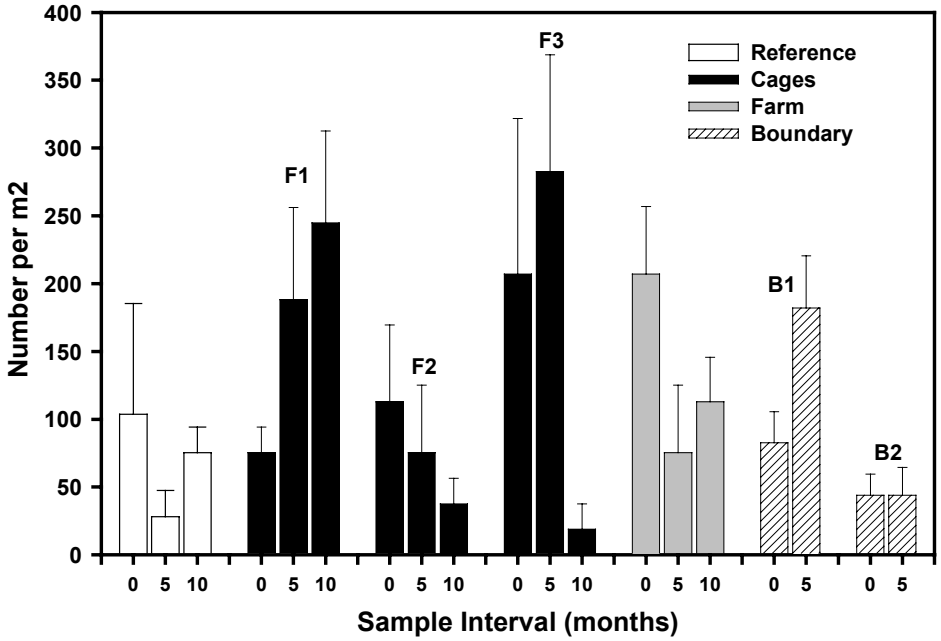


Fig. 4.20. Number per m^2 of echinoderms at Nubeena sites.

Mollusc numbers were also higher at the cage sites (Table 4.7) largely as a result of the greatly increased numbers at 0-F1 (Fig. 4.21). Two-way ANOVA of the molluscan

abundance data showed a significant site/time interaction ((N = 75, df = 6, F = 2.949, p = 0.014 for all sites at 0 and 5 months; N = 51, df = 8, F = 4.048, p=0.002 for Cage, Farm and Reference groups at 0, 5 and 10 months). Pairwise comparisons indicated that site 0-F1 was significantly different to all other sites except 5-R, 10-R, 5-F2, 5-F3, 0-F 35 m and 0-B1.

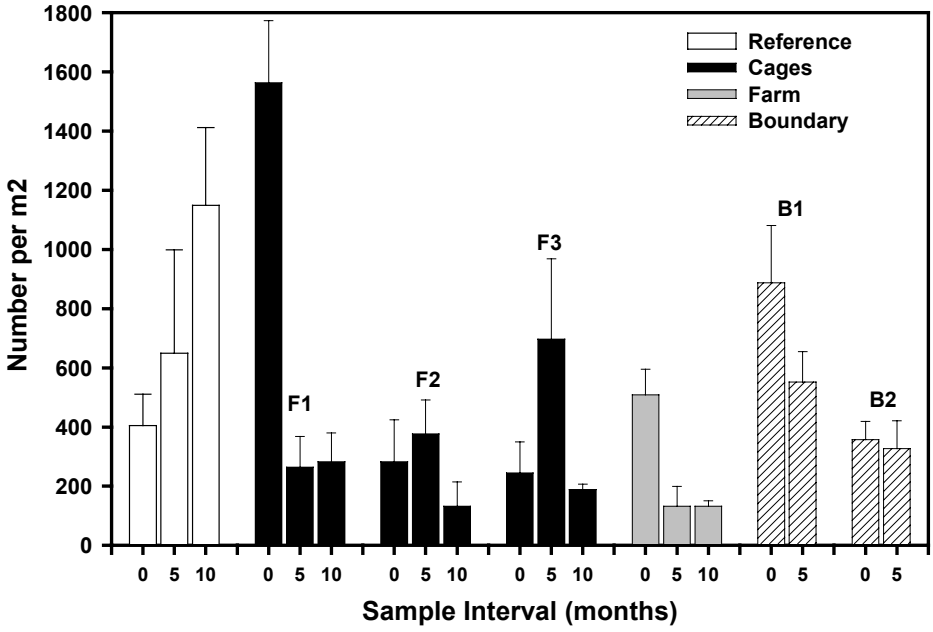


Fig. 4.21. Number per m^2 of molluscs recorded at sites at Nubeena.

Univariate indices: The results from the univariate indices of environmental change were equivocal (Table 4.8). The lowest mean number of species recorded in the "a priori" groups was at the farm F35 m group and the highest at the boundary group (Table 4.8). Overall, the highest mean number of species occurred at the B1 transect, and the lowest at the F2 site. However, more detailed examination of the temporal data indicates the number of species recorded in each a priori group was very variable (Fig. 4.22). All sites except reference sites showed a decreased number of species at 5 months, and an increase at the 10 month sampling at the farm and cage sites. However, these differences were not significant.

Table 4.8. Univariate Diversity Indices for the "a priori" defined groups at Nubeena.

	No.of Species		No.Individuals		Shannon		Inv.Simpson	
	mean	se	mean	se	mean	se	mean	se
R1	25	3.2	3509	549.3	2.62	0.20	12.52	2.72
R2	20	3.3	1780	324.8	2.75	0.10	13.69	1.63
Reference	**23**	**2.3**	**2817**	**412.0**	**2.67**	**0.12**	**12.98**	**1.75**
B1	36	3.7	3436	244.4	3.23	0.10	19.42	1.77
B2	10	1.4	2524	206.2	2.75	0.09	15.20	1.27
Boundary	**27**	**2.2**	**3015**	**176.5**	**2.99**	**0.08**	**17.31**	**1.13**
Non-Cage (Ref & Boundary)	**25**	**1.8**	**2975**	**168.3**	**2.49**	**0.11**	**12.55**	**1.03**
F1	19	2.8	33628	14056.0	1.89	0.33	6.78	1.89
F2	12	1.5	26183	11229.1	1.41	0.27	3.88	1.17
F3	17	1.7	14815	8543.5	2.08	0.23	7.47	1.66
Cage	**16**	**1.3**	**24875**	**6556.3**	**1.79**	**0.16**	**6.04**	**0.94**
Farm (F.35m)	**13**	**1.9**	**1695**	**260.6**	**2.19**	**0.31**	**9.71**	**1.67**

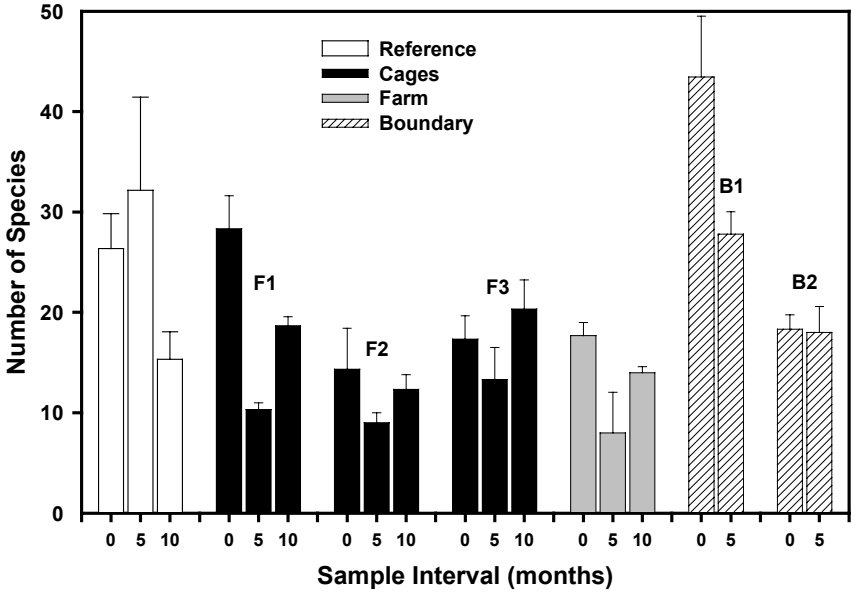


Fig. 4.22. Mean number of species recorded for sites at Nubeena.

The highest numbers of individuals recorded were clearly at the sites next to cages (Table 4.8, Fig. 4.23) and in particular at the 10 month survey (Fig. 4.23). The mean number recorded at the cage locations over the sampling period was 24,875, whilst at the non-cage sites this was reduced to 2,975. Significant differences were found between sites over time using two-way ANOVA ($N = 72$, $df = 7$, $F = 7.664$, $p<0.001$ for all sites at 0 and 5 months; $N = 45$, $df = 8$, $F = 10.525$, $p<0.001$ for sites F1, F2, F3, F 35 m and R1 at 0, 5 and 10 months). Pairwise comparisons showed that the "a priori" cage group was significantly different from the other groupings at all times and that the three cage sites F1, F2 and F3 were significantly different from all others at 10 months.

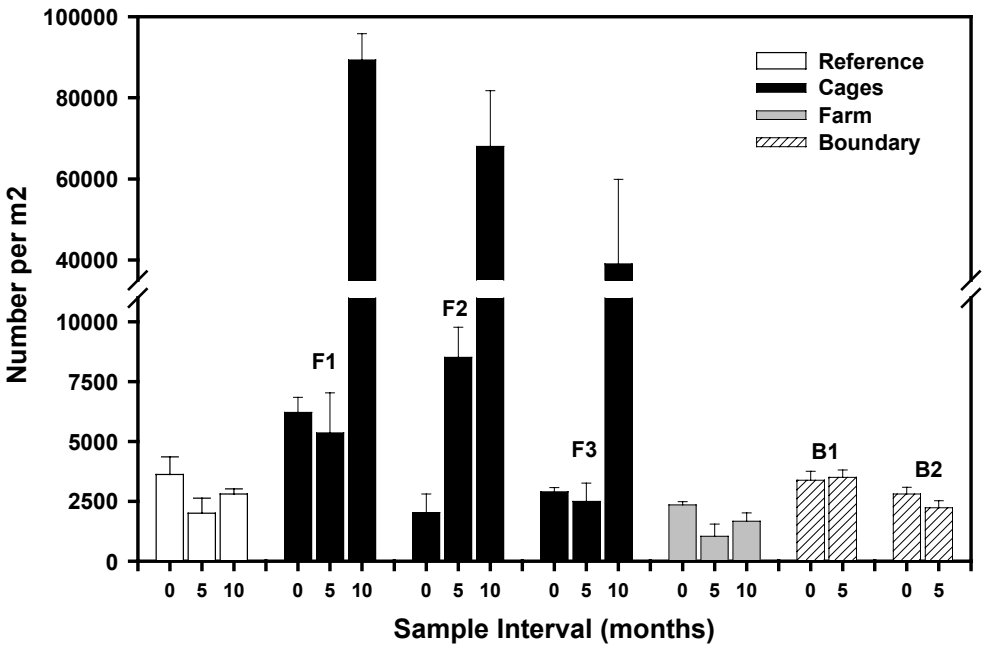


Fig. 4.23. Mean number of individuals recorded for all sites at Nubeena.

The Shannon diversity indices were lowest at the cage sites (Table 4.8). Index values less than 2 were noted from cage, farm and reference locations but not from any of the boundary sites (Fig. 4.24). Index values less than 1 were only obtained at the F1 and F2 sites (next to the cage) at 10 months (Fig. 4.24). ANOVA showed a significant site/time interaction ($N = 80$, $df = 3$, $F = 13.595$, $p<0.001$ for Cage, Farm, Boundary and Reference groups at 0 and 5 months: $N = 59$, $df = 2$, $F = 8.306$, $p = 0.001$ for Cage, Farm and Reference groups at 0, 5 and 10 months). Shannon diversity index values declined over time at both reference and cage sites, but this decrease was much greater at the cage sites. After 10 months the indices at the cage sites were significantly lower than at the start of the study, in particular values at sites F1 and F2 were significantly lower than at any other sites.

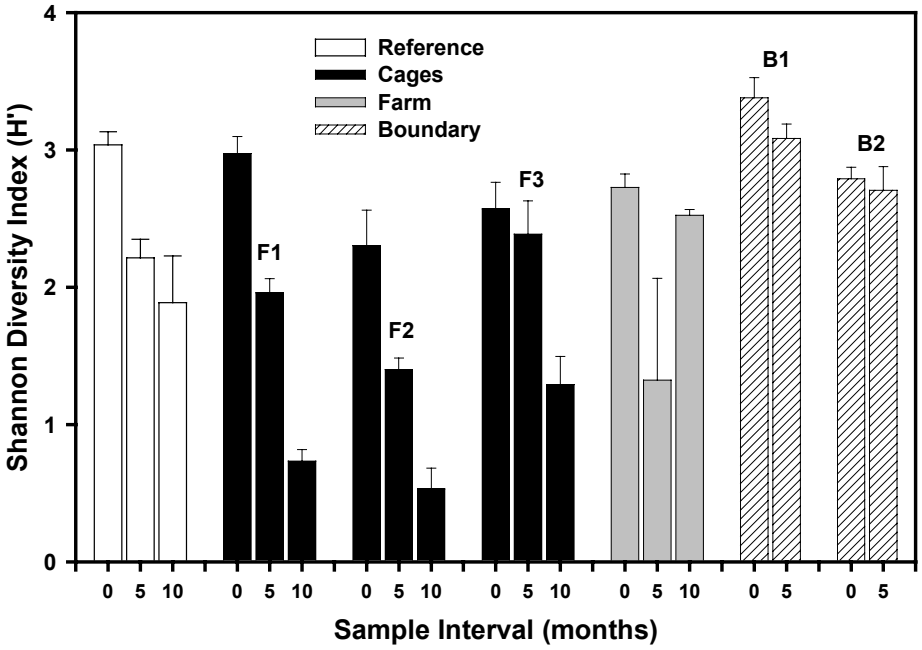


Fig. 4.24. Shannon Diversity Index for sites at Nubeena.

The mean Inverse Simpson Index values were consistently highest at the boundary sites (Table 4.8, Fig. 4.25). At reference and cage sites this index dropped over time, although to lower levels at the cage sites (Fig. 4.25). ANOVA showed significant differences between sites and over time, but the interaction term was not significant. Pairwise comparisons showed that cage group values were significantly lower than at either boundary or reference groups (Table 4.8, N = 80, df = 3, F = 10.002, $p<0.001$ for all sites at 0 and 5 months; N = 59, df = 2, F = 3.509, p = 0.038 for Cage, Farm and R1 sites at 0, 5 and 10 months). Each of the cage sites showed a clear decline in this index over time, with the values being significantly lower at all of the cage sites at the 10 month survey.

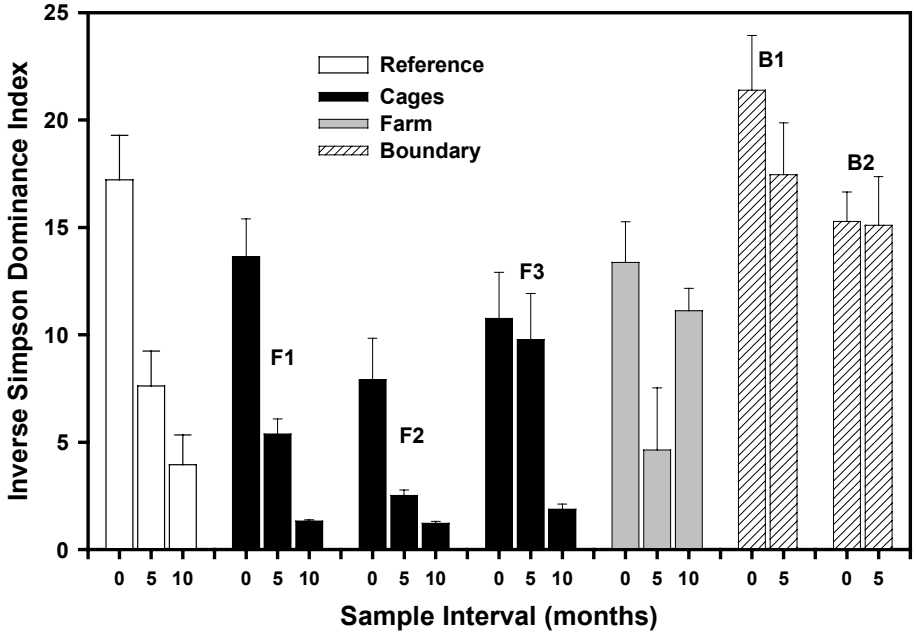


Fig. 4.25. Inverse Simpson Dominance Index for sites at Nubeena.

Hideaway Bay

A total of 315 species and 41,065 individuals were identified from Hideaway Bay. The mean number of annelids m^{-2} was markedly higher at F 0 m next to the cage of salmon (2,530 m^{-2}), than at all other sites where it ranged from 205 m^{-2} (reference sites) to 928m^{-2} (F 60 m) (Fig. 4.26, Table 4.9). *Capitella* sp. (MoV2558) was encountered at all site groups, although the greatest abundance consistently occurred at the F 0 m site. Two-way ANOVA indicated significant interaction between sites and times ($N = 313$, $df = 30$, $F = 12.600$, $p<0.001$ for annelids; $N = 313$, $df = 30$, $F=21.515$, $p<0.001$ for *Capitella* sp. (MoV2558)). Pairwise comparison showed that there were significantly higher numbers m^{-2} of both annelids and *Capitella* sp. (MoV2558) at the site next to the cage (F 0 m) at the 9 and 11 month surveys than at any other site or time. *Capitella* sp. (MoV2558) abundance was also clearly elevated at the F 10 m site, particularly at the 9 month sample time. Annelid abundance at the F 60 m site was also significantly different from all other groups except F 10 m.

Table 4.9. Mean, minimum and maximum numbers of individuals m^{-2} within the major faunal groups for the main site groupings at Hideaway Bay.

		Annelida	Crustacea	Mollusca	Echinodermata	*Capitella* sp.
REFERENCE	**mean**	**205**	**65**	**187**	**106**	**1**
	min	0	0	0	0	0
	max	1 243	339	734	282	30
INSHORE	**mean**	**443**	**202**	**965**	**73**	**9**
	min	0	0	0	0	0
	max	1 864	847	6 836	282	169
OFFSHORE	**mean**	**235**	**75**	**322**	**144**	**4**
	min	30	0	0	0	0
	max	904	356	1 156	621	169
F.0m	**mean**	**2 530**	**52**	**86**	**0**	**2 503**
	min	193	0	0	0	163
	max	5 837	397	222	0	5 822
F.10m	**mean**	**513**	**15**	**426**	**29**	**434**
	min	119	0	0	0	0
	max	2 563	59	1 585	193	2 533
F.35m	**mean**	**310**	**92**	**922**	**210**	**11**
	min	222	0	227	0	0
	max	489	237	1 807	400	59
F.60m	**mean**	**928**	**264**	**693**	**149**	**13**
	min	378	132	193	59	0
	max	1 200	489	1 304	252	74
NON-CAGE	**mean**	**284**	**107**	**466**	**117**	**5**
(Ref, Inshore &	min	0	0	0	0	0
Offshore)	max	1 864	847	6 836	621	169

Similar to Nubeena, annelid abundance was strongly correlated with *Capitella* sp. (MoV2558) abundance ($N = 313$, $r = 0.890$, $p = 0.01$). The large increases observed in annelid abundances were as a direct result of increases in the numbers of *Capitella* sp. (MoV2558).

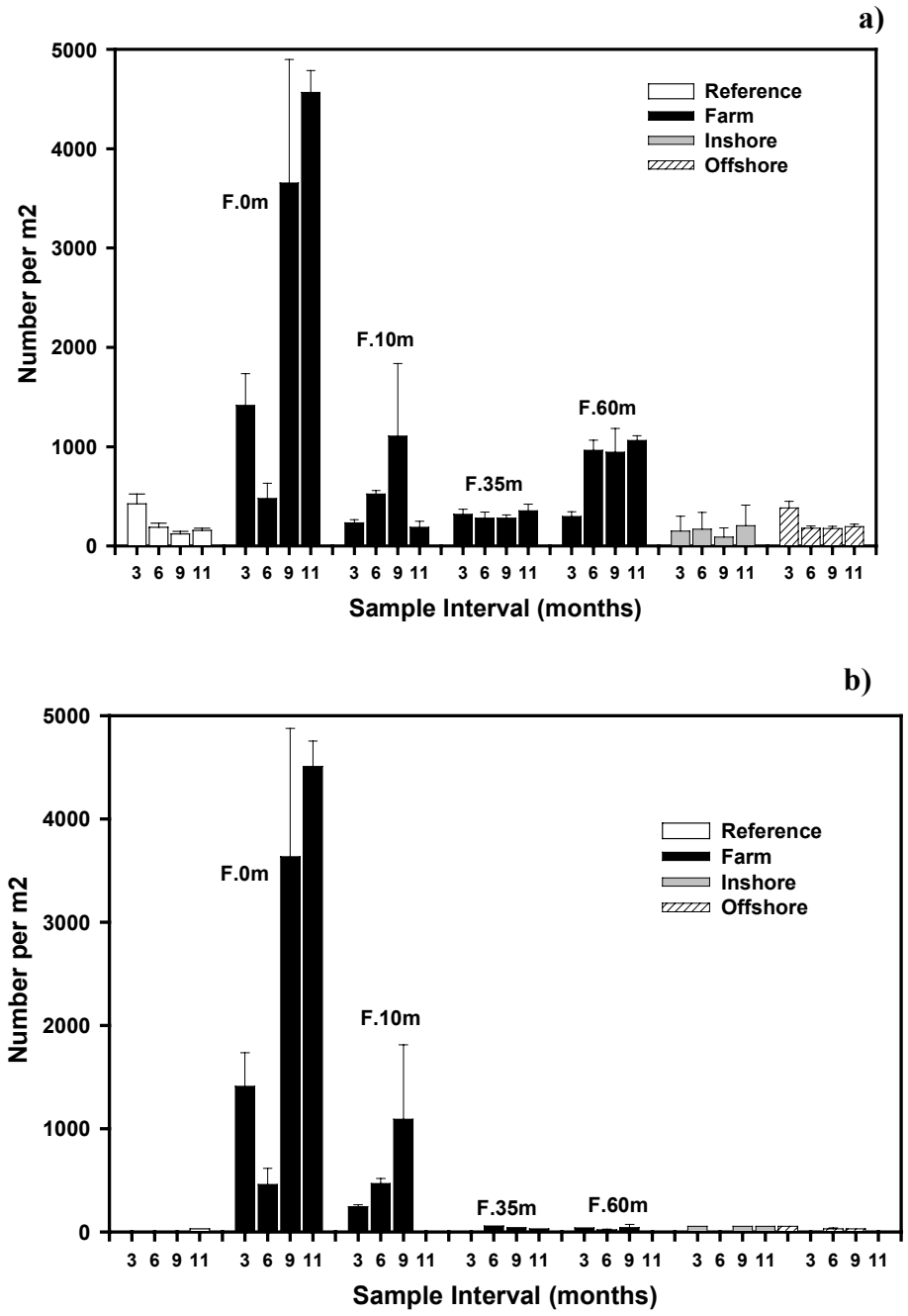


Fig. 4.26. Number m^{-2} of a) annelids and b) *Capitella spp* at Hideaway Bay sites.

Crustaceans were most abundant at the F 60 m site and at the inshore boundary transects at 6 months (Table 4.9, Fig. 4.27). Few crustaceans were recorded at the F 0 m and F 10 m sites, except at 3 months at F 0 m. ANOVA of the seven predetermined site groups indicated a significant site*time interaction (N = 313, df = 18, F = 5.711, $p<0.001$). Crustacean abundances largely declined at reference and F 0 m sites over time, but increased at F 35 m, and were highest at inshore boundary sites at 6 months and at F 60 m at 9 months. Variability between replicates was relatively high. Elevated crustacean numbers at F 60 m are likely to have occurred because this site was adjacent to a small area of rocky reef.

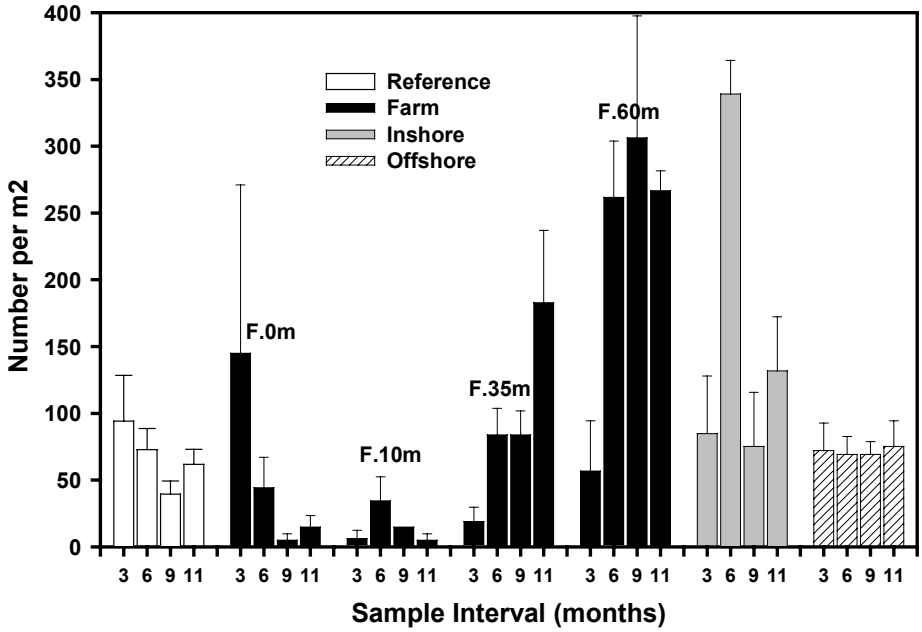


Fig. 4.27. Number per m^2 of crustacea at Hideaway Bay.

Echinoderms showed a varied distribution. They were conspicuously absent from the F 0 m site, however, they were also absent from several other sites, including some reference site replicates (Table 4.9, Fig. 4.28). Numbers were also relatively low at F10 m, but were highest at F 35m. ANOVA of the 7 predetermined site groupings showed a significant site*time interaction ($N = 313$, $df = 18$, $F = 2.635$, $p<0.001$). Greatest abundances occurred at F 35 m, followed by F 60 m sites (Fig. 4.28). There was no clear trend in abundance over time.

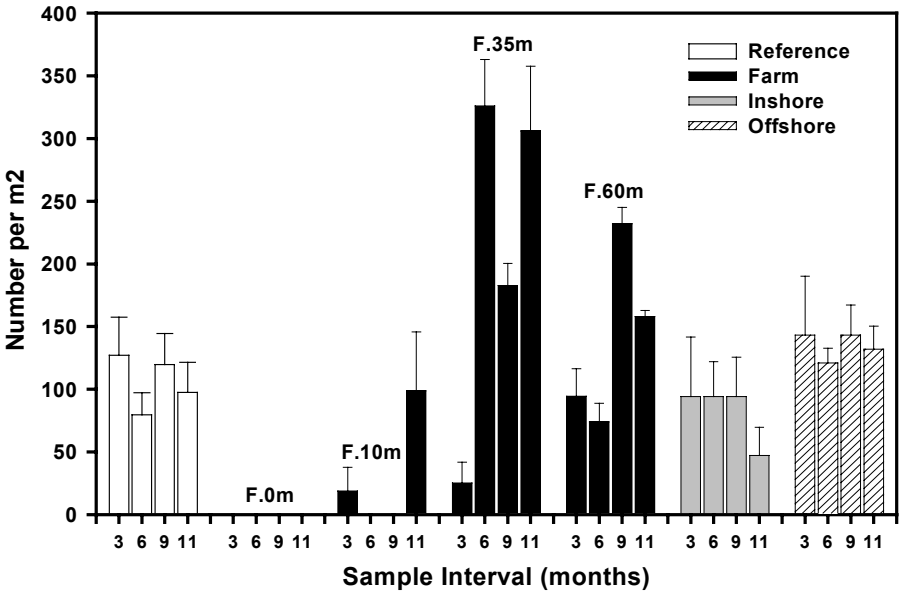


Fig. 4.28. Number per m^2 of echinoderms at Hideaway Bay.

Molluscs were generally fairly numerous at all sites except F 0 m (Table 4.9, Fig. 4.29). The abundance was particularly high at the inshore boundary transects and F 35 m sites. Two-way ANOVA indicated a significant site/time interaction for the 7 predetermined site groupings (N = 313, df = 18, F = 9.000, $p<0.001$) and pairwise comparisons suggested that overall, molluscs were significantly more abundant at both the inshore grouping and F 35 m site than at other sites.

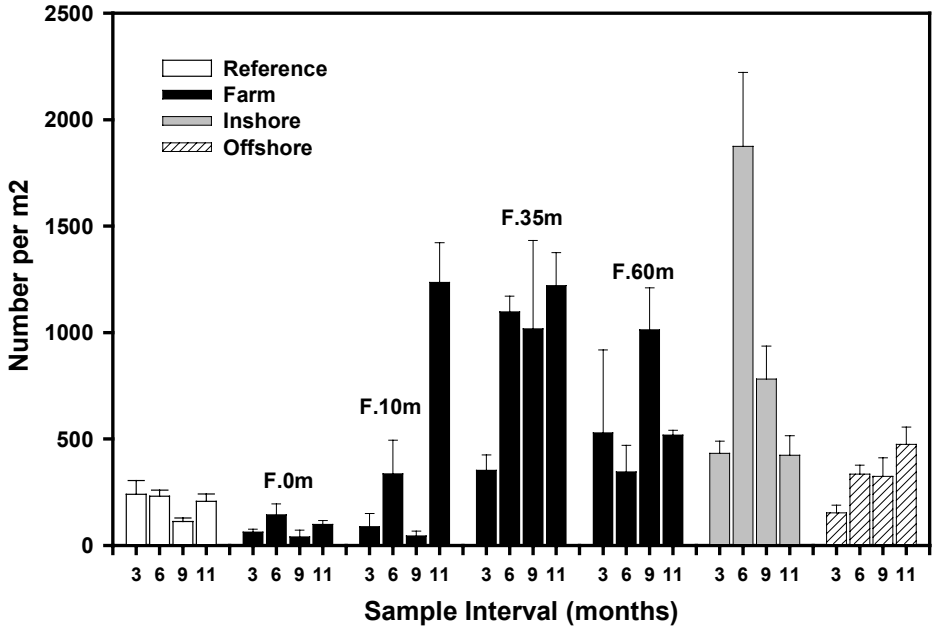


Fig. 4.29. Number m^{-2} of molluscs at Hideaway Bay.

Univariate indices: Hideaway Bay generally showed a similar pattern of results to those from the Nubeena salmon farm.

The lowest mean number of species occurred at F 0 m, but levels were also low at F 10 m and R4 (Table 4.10, Fig. 4.30). ANOVA of the 7 predetermined site groupings showed a significant interaction between site and time ($N = 313$, $df = 18$, $F = 4.257$, $p<0.001$). Much higher numbers of species were found at the F 60 m site and pairwise comparison showed that this site had significantly higher numbers of species than any other site groupings, except at 3 months. Sites R1 and F 35 m had the next greatest numbers of species, but overall only contained about 60% of the number of species recorded from F 60 m (Table 4.10, Fig. 4.30). Pairwise comparisons also showed that in general there were significantly more species at the F 35 m sites than at the F 0 m, F 10 m, reference and inshore sites. Similarly, in general species numbers were significantly lower at the F 0 m site than all other site groups except F 10 m.

Table 4.10. Univariate Diversity Indices at Hideaway Bay.

	No.of Species		No.Individuals		Shannon		Inv.Simpson	
	mean	se	mean	se	mean	se	mean	se
R1	19	2.1	839	136.7	2.55	0.10	10.02	0.92
R2	13	1.1	868	110.8	2.17	0.07	6.77	0.52
R3	12	1.3	536	60.5	2.06	0.15	6.68	0.74
R4	9	0.9	385	51.3	1.77	0.11	4.81	0.48
Reference	**14**	**0.9**	**685**	**60.5**	**2.18**	**0.06**	**7.30**	**0.45**
B1	14	1.1	2173	247.5	2.04	0.09	6.56	0.57
B2	10	0.6	1508	168.0	1.82	0.07	5.26	0.41
Inshore Boundary	**12**	**0.7**	**1840**	**152.8**	**1.93**	**0.06**	**5.91**	**0.36**
B3	13	0.9	864	74.7	2.09	0.06	6.55	0.47
B4	16	0.9	773	54.5	2.22	0.06	7.20	0.52
B5	16	0.5	770	50.4	2.17	0.05	6.28	0.49
B6	14	0.7	948	72.1	2.07	0.06	5.76	0.38
Offshore Boundary	**15**	**0.4**	**836**	**32.3**	**2.14**	**0.03**	**6.49**	**0.24**
Non-Cage (Ref & Boundary)	**14**	**0.3**	**1103**	**56.6**	**2.08**	**0.03**	**6.44**	**0.18**
F.0m	6	0.8	2564	593.8	0.59	0.15	1.60	0.21
F.10m	9	1.4	1011	219.0	1.05	0.16	2.26	0.34
F.35m	18	1.5	1500	218.4	1.99	0.09	4.85	0.57
F.60m	31	3.5	2040	234.2	2.77	0.17	12.68	2.18

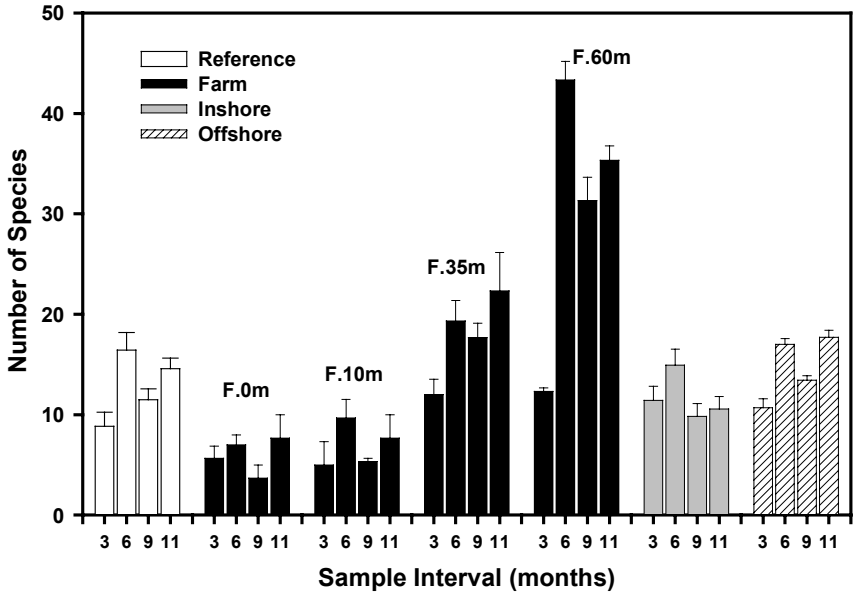


Fig. 4.30. Mean number of species recorded for sites at Hideaway Bay.

The greatest numbers of individuals were recorded from the F 0 m group (2,564) and the lowest in the reference group (R4 = 385, overall reference group = 685, Table 4.10), although there was considerable variability within these groups over time (Fig. 4.31). The highest mean number of individuals at any site was at the F 0 m site at the last sampling (4,696). Numbers generally increased over time at the cage sites but decreased at the reference sites. ANOVA of number of individuals from the seven predetermined site groupings indicated a significant site/time interaction (N = 313, df=18, F = 13.185, $p<0.001$) and the pairwise comparisons suggested that the abundance was significantly higher at the F 0 m site than at all other sites except F 60 m.

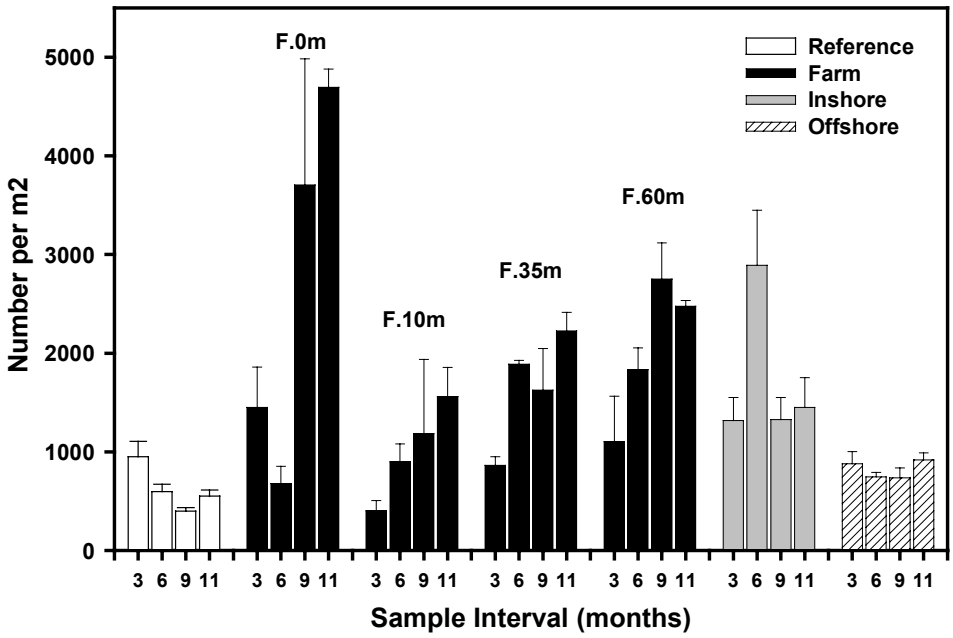


Fig. 4.31. Mean number of individuals recorded from sites at Hideaway Bay.

Most sites had a mean Shannon index >2 (Table 4.10, Fig. 4.32). Only sites in the F 0 m group had an index value <1, and F 10 m was the next lowest at 1.05 (Table 4.10). The highest Shannon index occurred at the F 60 m group. Two-way ANOVA of the site groupings showed a significant site and time interaction (N = 313, F = 2.508, p=0.001) and pairwise comparisons indicated that the later F 0 m samples were significantly lower than the reference, boundary, F 35 or F 60 m sites.

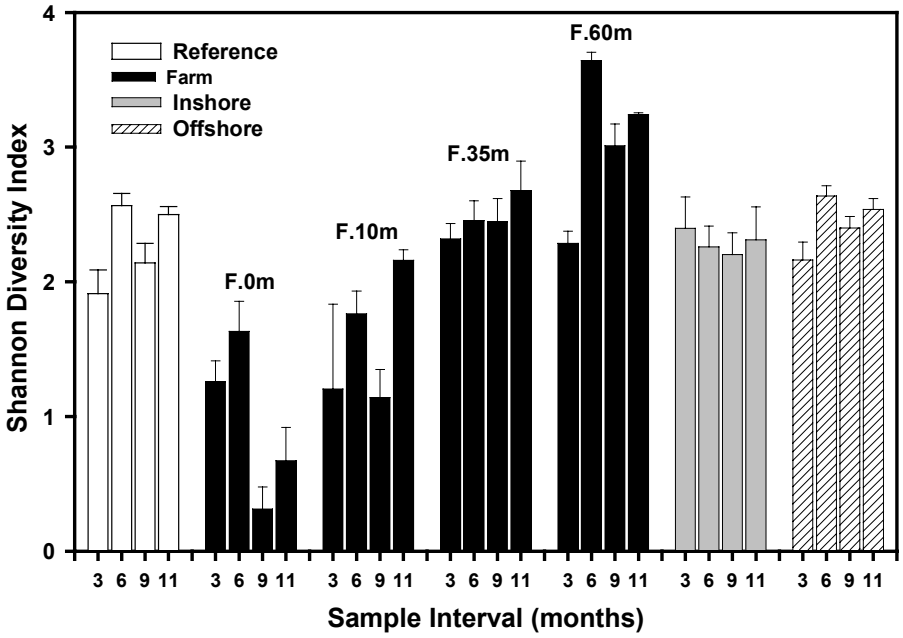


Fig. 4.32. Shannon diversity index for sites at Hideaway Bay.

The mean Inverse Simpson's Index was >6 at most sites, and overall was highest at F 60 m, and lowest at F 0 m and F 10 m (Table 4.10, Fig. 4.33). The interaction between site and time was significant (ANOVA: N = 313, df = 18, F = 4.483, p<0.001). Pairwise comparison indicated a significant reduction in this index at groups F 0 m and F 10 m compared to the other groups. Values less than 2 were only recorded at the site next to the cage (F 0 m) at 9 and 11 months.

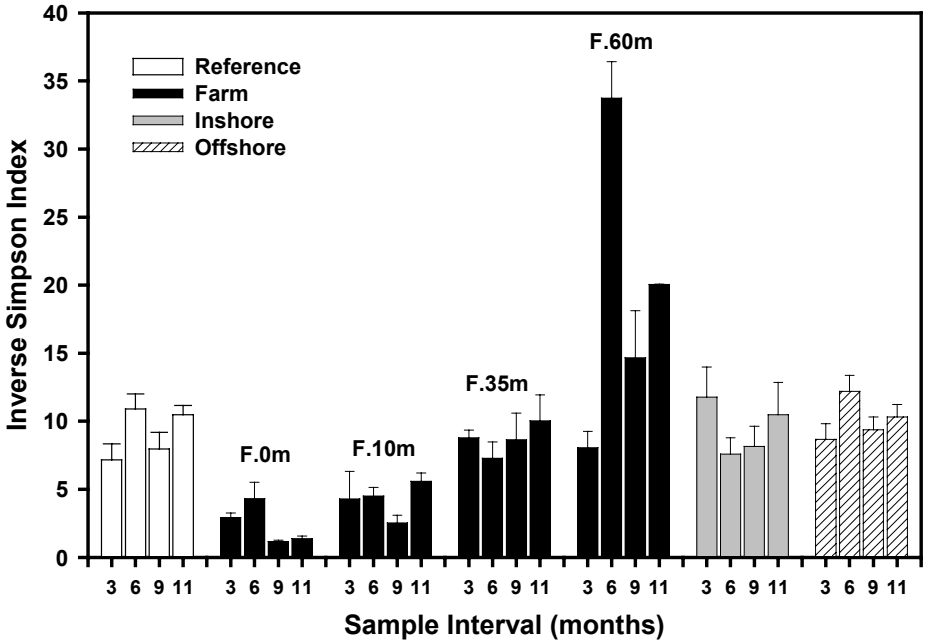


Fig. 4.33. Inverse Simpson dominance index for sites at Hideaway Bay.

4.3.5 Evaluation of level of taxonomic discrimination

Nubeena

Data from all sites investigated are included in the analysis. Multivariate analysis of the community structure of the fauna at Nubeena at species level clearly identified two community groups, separating at ~ 18% similarity (Fig. 4.34). One group contained all the reference and boundary sites, and the other group contained all the farm cage associated sites. This second group sub-divided into two smaller groups at ~ 26% similarity: Group 2a contained almost all sites next to cages at the 5 and 10 month surveys (except F3), whereas Group 2b contained the cage sites at the beginning of sampling, F3 at 5 months, and F 35 m at all sampling times (Fig. 4.34a). Group 1 sites were further divided at ~ 30% similarity into western (R1, B1) and eastern (R2, B2) community groups, indicating a spatial effect on the community structure. The MDS ordination plot (Fig. 4.34b) clearly shows increasing impact from organic enrichment as a curving progression across the plot from left to right.

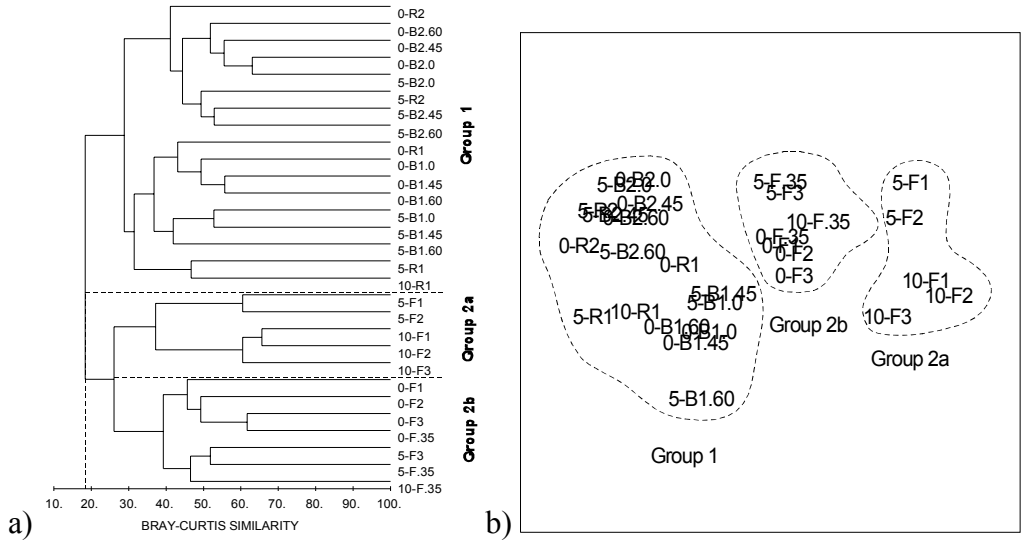


Fig. 4.34. Species level - a) cluster analysis plot and b) MDS plot (Stress-0.13) at Nubeena.

Increasing the level of taxonomic discrimination to family level, (Fig. 4.35), resulted in the identification of three groups with the same combination of sites as identified in the species level assessment. However, the order in which the groups separate changed. The primary dichotomy at family level, occurred at 20% similarity and distinguished the impacted farm cage sites (Group 1) from all other sites. The remaining, less impacted, farm cage sites were grouped with the boundary and reference sites, and were separated from them at the next cluster level (Group 2b). The MDS ordination plot (Fig. 4.35b) shows the groups to be positioned across the plot in a similar manner to that of species level.

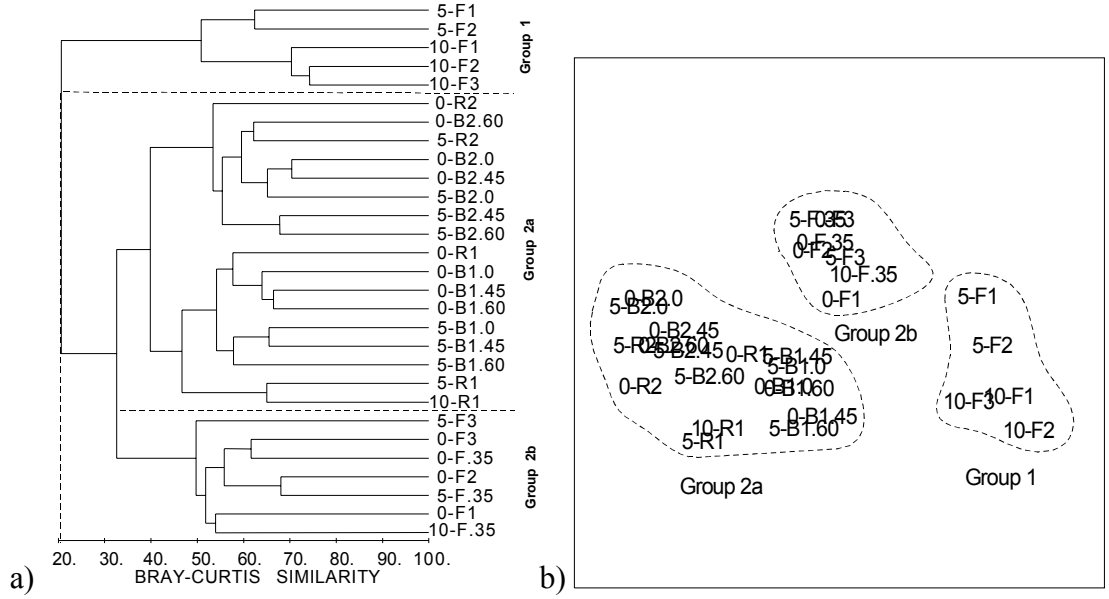


Fig. 4.35. Family level -a) cluster analysis plot and b) MDS plot (Stress=0.11) at Nubeena.

At order level (Fig. 4.36) the sites no longer clearly formed the same three groups as described above. The sites likely to have experienced the greatest impact (10-F1, 10-

F2, 10-F3) were distinguished at 28% similarity in Group 1 (Fig. 4.36a). Most of the remaining sites next to farm cages were in Group 2b, except for site 0-F1 which was associated with the unimpacted community group. The MDS ordination plot (Fig. 4.36b) still showed a distribution pattern across the plot similar to that of both species and family level. Increasing the level of identification to class (Fig. 4.37) resulted in a marked deterioration in ability to discern the levels of impact. The most impacted sites (10-F1, 10-F2, 10-F3) were, nevertheless, still clearly distinguishable at 38% similarity and there was still evidence of gradation in the farm cage sites, with Group 2b containing most of the remaining cage sites. In the MDS plot the most disturbed sites were clearly separated from all others. At phylum level (Fig. 4.38), while the most impacted farm sites were still identifiable (10-F1, 10-F2, 10-F3 and 5-F2), the subsequent gradation of the farm sites was no longer evident. The division of the second cluster group appeared to be mainly related to the spatial location of the sites rather than level of impact. The farm transect was at the western end of the lease and these cage sites were distributed with the B1 samples.

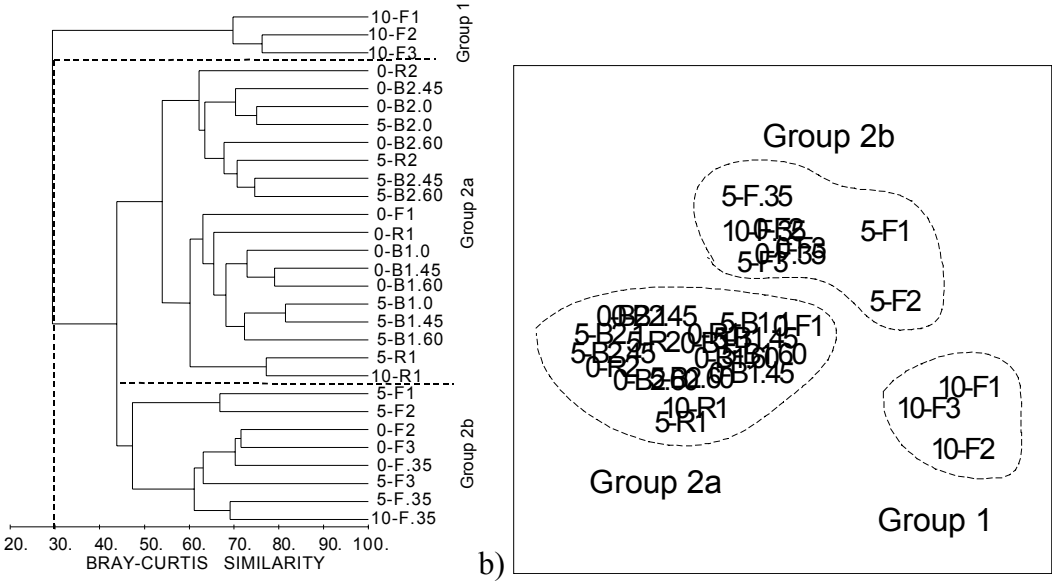


Fig. 4.36. Order level -a) cluster analysis plot and b) MDS plot (Stress = 0.12) at Nubeena.

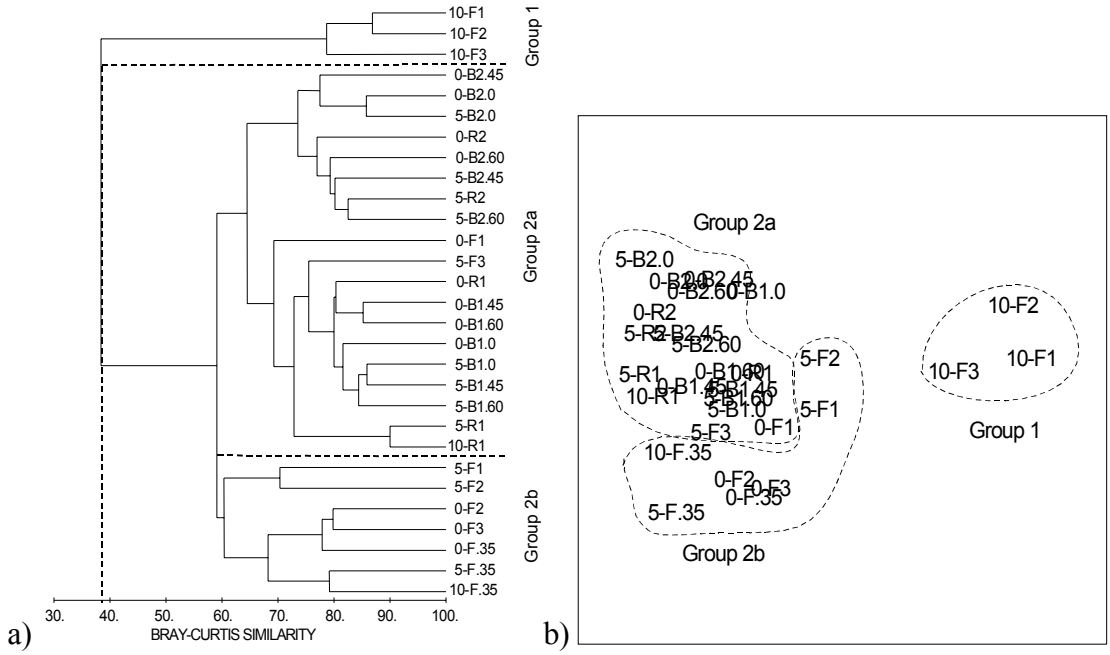


Fig. 4.37. Class level -a) cluster analysis plot and b) MDS plot (Stress=0.12) at Nubeena.

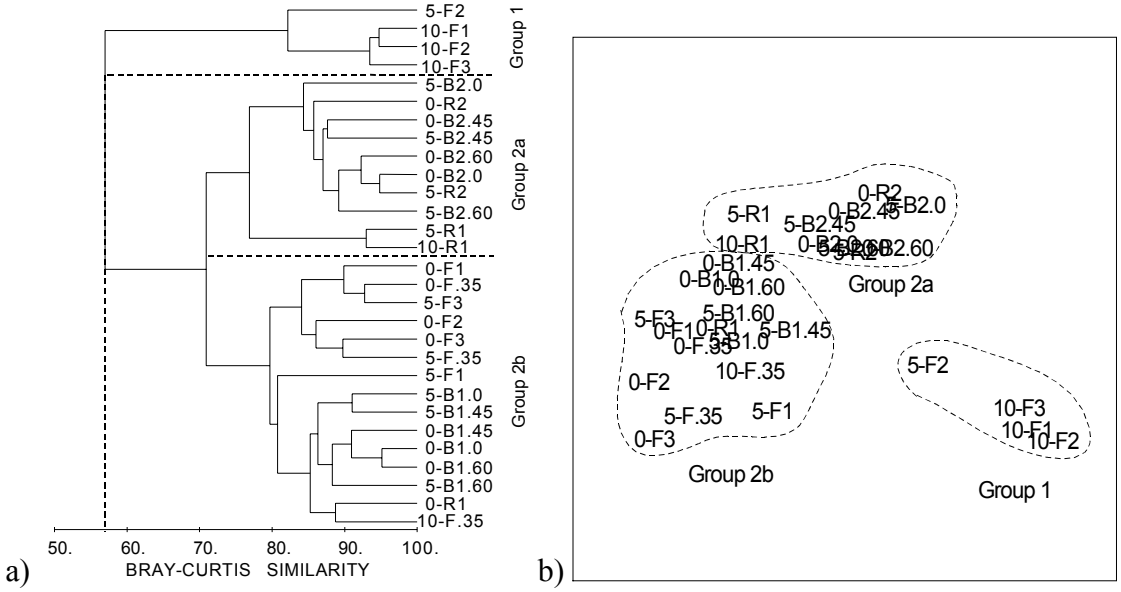


Fig. 4.38. Phylum level -a) cluster analysis plot and b) MDS plot (Stress=0.08) at Nubeena.

Ranked similarity matrices for each of the taxonomic levels were compared with the species level assessment by RELATE analysis (Table 4.11). Even at phylum level there was a strong relationship between the similarity matrices. However, the global RHO (Harmonic Spearman rank correlation coefficient) decreased at each consecutive taxonomic level. The correlation coefficient for species and family level had the highest value indicating a very strong relationship between the two similarity matrices.

Table 4.11. Comparison of higher taxonomic groups with species level identification by RELATE analysis at Nubeena.

Taxonomic Level	Global RHO	Significance
Family	0.913	<0.0001
Order	0.824	<0.0001
Class	0.698	<0.0001
Phylum	0.664	<0.0001

Diversity indices at Nubeena for the varying levels of taxonomic discrimination are shown in Table 4.12. At all taxonomic levels the mean number of taxa in each group decreased from unimpacted through minor to major impact. However, the differences between impact groups decreased from species through to phylum level. Thus, at species level differences between impact groups were obvious, with the mean number of taxa in the unimpacted group being almost double that of the major impact group. At family level, the unimpacted group still clearly contained more taxa than the moderate and major impact groups, but by phylum level there was little difference between impact groups. The proportion of families that consisted of a single species ranged from 78% to 81% across the impact groups, thus there was a high likelihood that family level would mimic species level identification.

Table 4.12. Mean numbers of taxa, Shannon Diversity Index and Inverse Simpsons Index at each taxonomic level of species, family, order, class and phylum at Nubeena. Sites are displayed in their species level multivariate groups.

	Species	Family	Order	Class	Phylum
References					
Mean No of Taxa	37	28	20	10	7
Mean Shannon Index	2.89	2.44	2.13	1.41	1.21
Mean Inverse Simpson	15.46	7.18	4.95	2.83	2.58
Unimpacted (Group 1)					
Mean No of Taxa	44	34	22	11	7
Mean Shannon Index	3.27	2.67	2.26	1.46	1.25
Mean Inverse Simpson	19.70	9.20	6.15	2.96	2.76
Moderate (Group 2b)					
Mean No of Taxa	30	22	15	9	5
Mean Shannon Index	2.91	2.40	2.09	1.73	1.21
Mean Inverse Simpson	13.12	7.48	5.73	4.54	2.82
Major (Group 2a)					
Mean No of Taxa	25	19	14	9	5
Mean Shannon Index	1.42	0.79	0.70	0.44	0.39
Mean Inverse Simpson	2.73	1.73	1.62	1.40	1.38

Mean Shannon Diversity indices were highest at the unimpacted sites, slightly lower at moderate impact, and much lower at the major impact sites across all taxonomic levels (except at class level where the index was higher at moderate than unimpacted sites). The Inverse Simpsons Index also clearly distinguished the three impact groupings at

species and family level. However, by Order level the mean of this index was similar between unimpacted and moderate impact sites, and at class and phylum level it was higher at the moderate impact than unimpacted sites. Highly impacted sites had a much lower Inverse Simpsons Index than other sites at all taxonomic levels, although the difference between impact groups was much more obvious at species level than at higher levels of classification.

Hideaway Bay

Species level multivariate assessment of the community structure at Hideaway Bay (Fig. 4.39) distinguished the sites next to the cage and 10 m distant. The MDS ordination plot (Fig. 4.39b) shows that Groups 1 and 2 are close, although they were distinguished at an overall similarity level of only 17%. Group 2 sites further divided at an overall similarity level of 22%. The pattern of site separation in group 2 (single sites or small groups of sites separating from the main group) is generally indicative of a gradual change in the community structure rather than major changes.

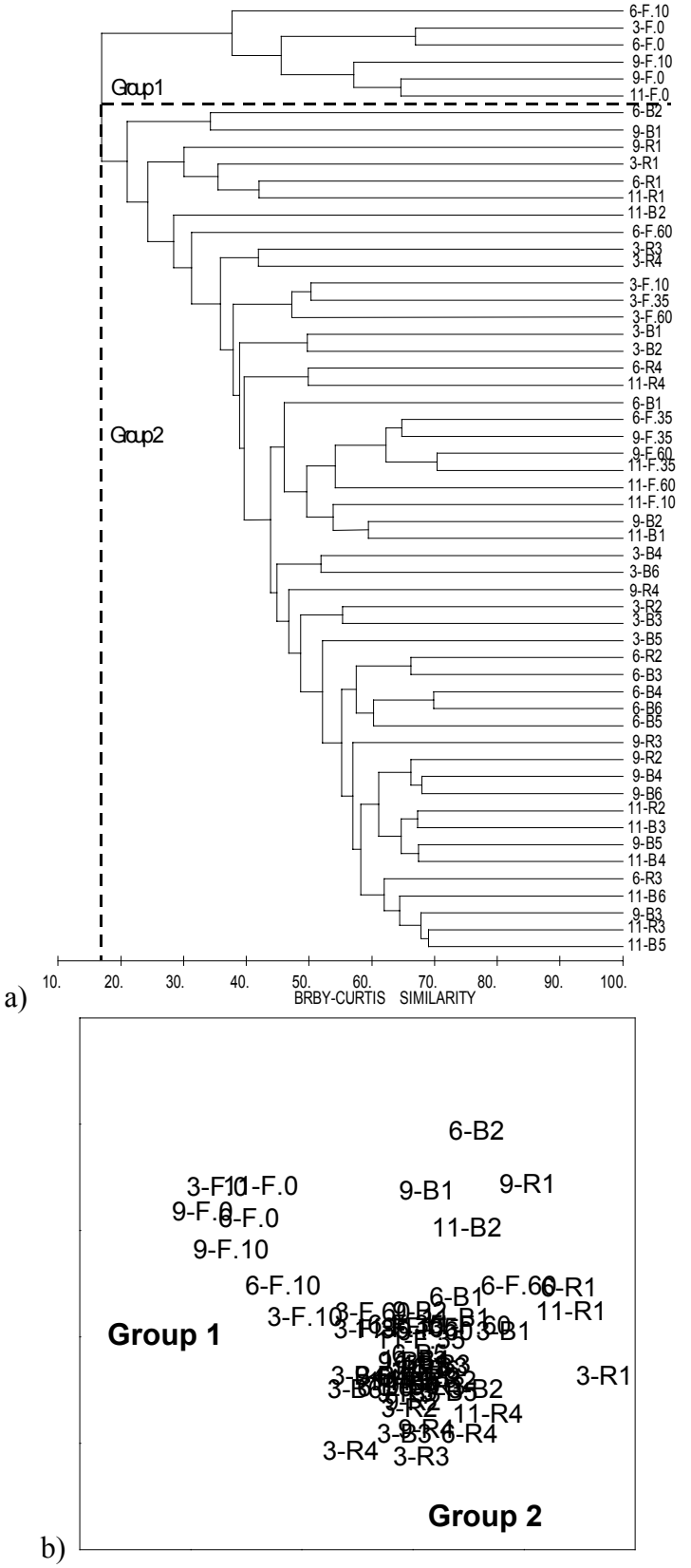


Fig. 4.39. Species level -a) cluster analysis plot and b) MDS plot (Stress=0.18) at Hideaway Bay.

Family and order level discrimination (Fig. 4.40 and Fig. 4.41) distinguished exactly the same sites within Group 1 as species level identification. However, the overall

similarity level at which the groups separated was higher with each increase in taxonomic level. At class level (Fig. 4.42) the number of sites in Group1 was reduced, and the separation of Group 1 from Group 2 was much more clearly defined. At phylum level the sites in Group 1 again included the site 10 m from the cage at 9 months as well as the sites next to the cage (Fig. 4.43).

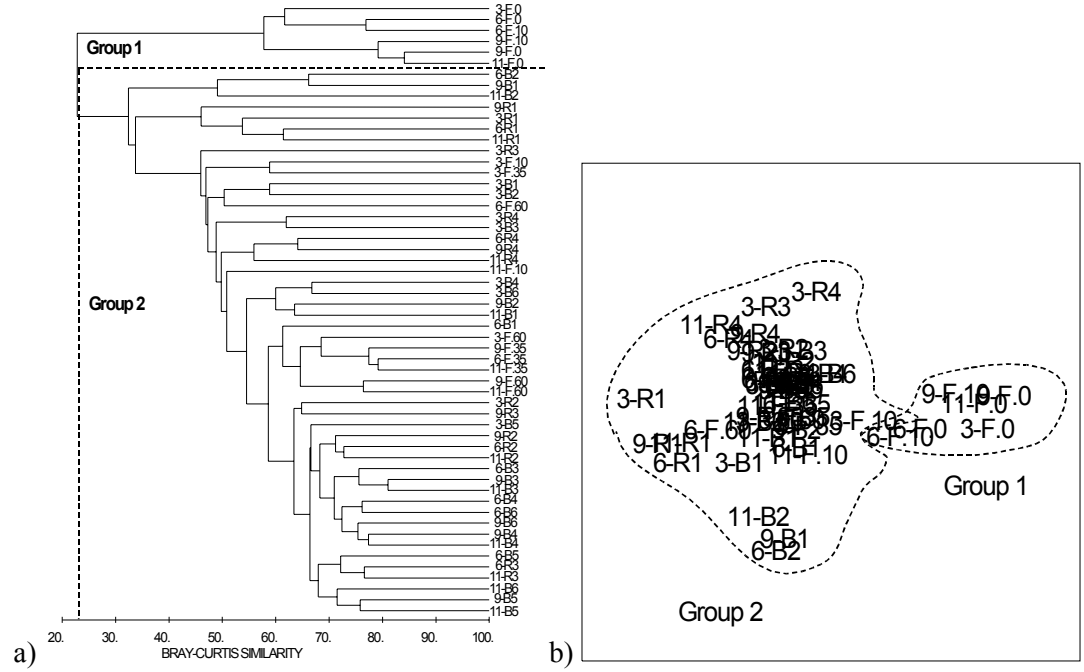


Fig. 4.40. Family level -a) cluster analysis plot and b) MDS plot (Stress=0.16) at Hideaway Bay.

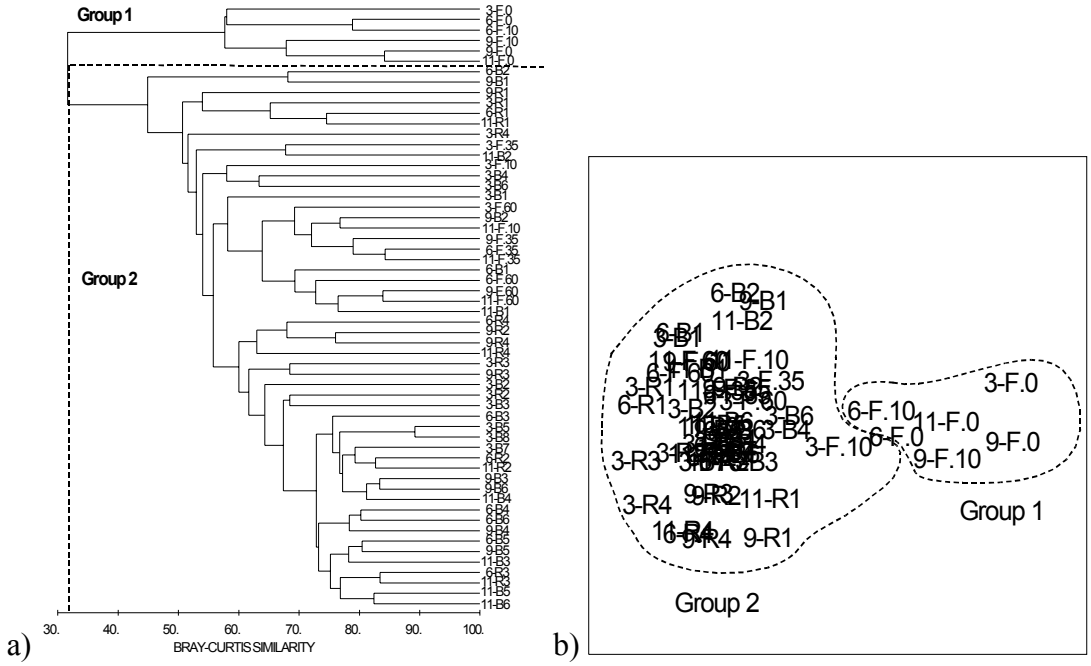


Fig. 4.41. Order level -a) cluster analysis plot and b) MDS plot (Stress=0.18) at Hideaway Bay.

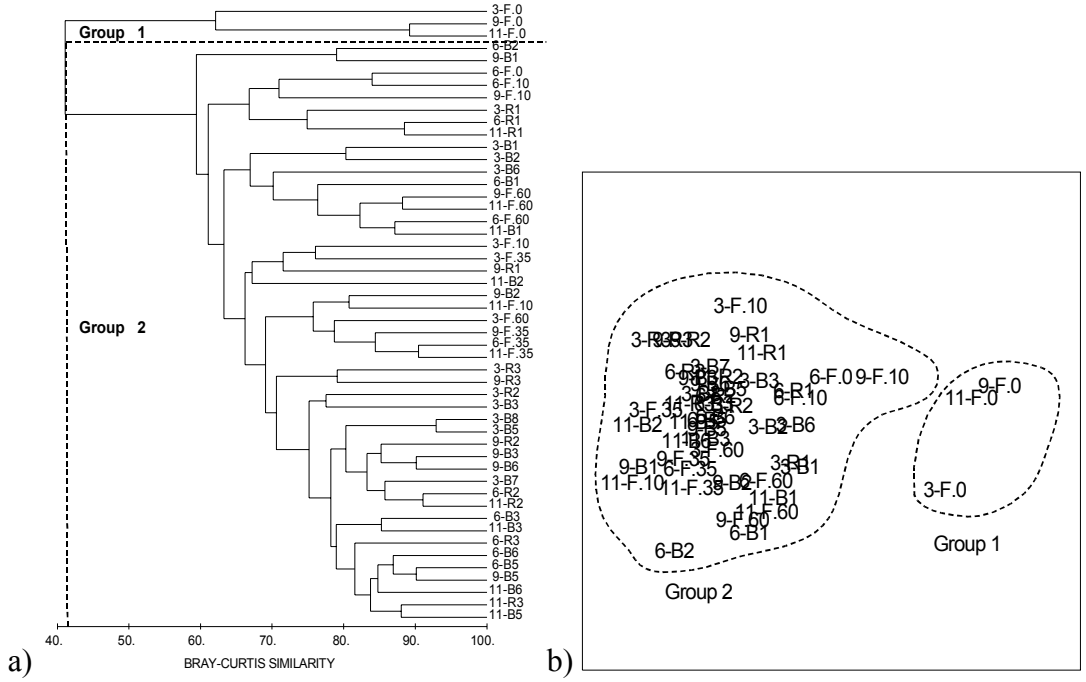


Fig. 4.42. Class level -a) cluster analysis plot and b) MDS plot (Stress=0.16) at Hideaway Bay.

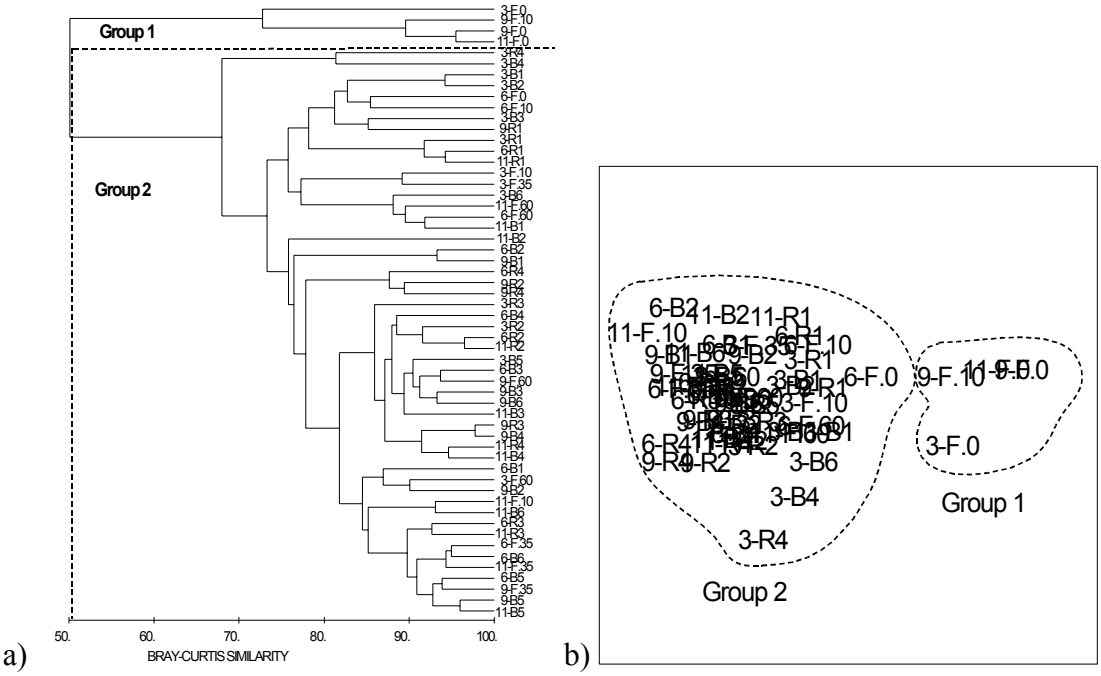


Fig. 4.43. Phylum level -a) cluster analysis plot and b) MDS plot (Stress=0.14) at Hideaway Bay.

Similar to Nubeena, RELATE analysis of the Hideaway Bay data (Table 4.13) indicated a significant relationship between species level identifications and those resulting from all higher levels of identification. The global RHO correlation value decreased at each increasing taxonomic level; family level had the highest value and therefore the closest relationship with species level. The similarities between the matrices were apparent in

the cluster analysis dendrograms (Fig. 4.39 – 4.43), where the individual sites generally maintained very similar positions at all the taxonomic levels.

Table 4.13. Comparison of higher taxonomic groups with species level identification by RELATE analysis at Hideaway Bay.

Taxonomic Level	Global RHO	Significance
Family	0.940	<0.0001
Order	0.860	<0.0001
Class	0.718	<0.0001
Phylum	0.703	<0.0001

Similar patterns in the diversity measures were found at Hideaway Bay to those at Nubeena, with only the most impacted sites being clearly distinguished. The difference in number of taxa found at major compared with minor impact sites decreased with increasing taxonomic group, from twice as many species at minor compared to major impact sites to only slightly more than one taxa difference at phylum level. At family level 92% of fauna found in the impact group belonged to families with only one species, compared with an average of 70% in the unimpacted group.

Table 4.14. Mean numbers of taxa, Shannon Diversity Index and Inverse Simpson Index at each taxonomic level of species, family, order, class and phylum at Hideaway Bay. Sites are displayed in their species level multivariate groups.

	Species	Family	Order	Class	Phylum
References					
Mean No of Taxa	25	22	16	8	5
Mean Shannon Index	3.03	2.46	2.20	1.59	1.31
Mean Inverse Simpson	18.84	8.29	6.61	3.99	3.26
Major Impact (Group 1)					
Mean No of Taxa	12	12	11	7	4
Mean Shannon Index	1.72	0.70	0.67	0.58	0.51
Mean Inverse Simpson	4.55	1.60	1.58	1.52	1.50
Minor/Unimpacted (Group 2b)					
Mean No of Taxa	27	22	16	9	6
Mean Shannon Index	3.08	2.34	2.13	1.59	1.28
Mean Inverse Simpson	19.34	7.39	6.20	3.85	3.05

At species level, both the Shannon and Inverse Simpsons Indices clearly separated the major impact group from the minor impact. However, the difference between the groups decreased with increasing taxonomic level, so that it became increasingly difficult at higher levels of classification to categorise sites by level of impact.

4.4 Discussion

The initial benthic fauna at each of the two salmon farms was different, and corresponded to the different environmental conditions, especially substrate type, at the two farms (described in Chapter 2). The fauna at Nubeena was largely characteristic of previously described fauna from sandy marine coastal environments around Tasmania (Edgar *et al.*, 1999; Macleod, 2000). Similarly, the fauna at the Hideaway Bay site was generally consistent with that previously found in soft sediment estuarine areas (Edgar *et al.*, 1999; Macleod, 2000). In both cases the foremost taxa was annelida; a large proportion of which were surface deposit and/or suspension feeding polychaetes. However at Nubeena, crustaceans made up a sizeable component of the fauna whilst at Hideaway Bay molluscs, proportionally, made a greater contribution. Only at the sites directly at the cages was a faunal community encountered which could be considered characteristic of organic enrichment (Pearson and Rosenberg, 1978; Brown *et al.*, 1987; Karakassis *et al.*, 1999).

The communities at both Nubeena and Hideaway Bay were clearly separated by both multivariate analysis and univariate indices into groups that could be directly related to various levels of organic enrichment. Major to severely impacted sites were clearly distinguishable using all assessment techniques. In particular, they were largely distinguished from other sites by very high abundances of *Capitella sp.* (MoV 2558), a species complex well recognised as being associated with high levels of organic enrichment. These severely impacted sites were identified next to cages of salmon at both farms, and at Hideaway Bay included sites 10 m from the edge of the cage at most sampling times. Densities of *Capitella sp* were markedly reduced at all other sites. The increase in *Capitella sp* at the cage sites was generally greater than two orders of magnitude at both farms.

Classification of sites as minor or moderately impacted was not as clear, and the analytical techniques varied sometimes in their classifications, particularly with respect to unimpacted and minor impact sites. At these sites it was useful to assess the benthic fauna using both multivariate and univariate measures in order to give a clearer overall picture of the level of impact.

The extent and degree to which the impact extended beyond the cages appeared to differ at the two farms. Densities of the organic indicator species *Capitella sp* were much higher next to cages at Nubeena than at Hideaway Bay which might suggest that the impact was greater at this site. However, the differences between the two farms in the benthic response to organic enrichment may have also been influenced by the different environmental conditions at the sites. Rosenberg (1976) suggested that estuarine ecosystems have a greater natural predisposition to organic enrichment than fully marine environments. Similarly Woodward *et al.* (1992) in their study of salmon farming in the Huon estuary suggested that coastal areas are less well adapted than estuarine systems for organic loading. Thus, the fauna at the Hideaway Bay site, as a result of the elevated background levels of organic material and finer sediment characteristics, may be better adapted to the lower sediment oxygen regimes than the fauna at Nubeena.

The effects measured 35 m from the edge of the cage also differed between the two farms. The Nubeena site 35 m from the edge of a cage was more similar to the heavily

organically enriched cage sites than to the unimpacted sites, and was classified as moderately impacted. In contrast, at Hideaway Bay the site 35 m from the cage edge was more closely associated with the deeper water reference and boundary transects than sites next to the cage. This supports the hypothesis that the Hideaway Bay site was better adapted to assimilate organic material, and this may also be affected by the differing current flow and dispersion dynamics of the sites.

The effect of the differing background environmental conditions was also evident in the grouping of sampling sites at Hideaway Bay. The vertical gradation of the Hideaway Bay sites in Groups 1a and 1b on the ordination plot (Fig. 4.6) suggests that these groups were differentiated along a different axis from that associated with the cage effects. This axis most likely reflects changes in other environmental factors, and the coarser sediments that occur at the inshore sites may be an important factor.

Several other studies on the spatial effects of organic enrichment from salmon cages have found that the impact is restricted to within 30 m of the edge of the cage. For example, Brown *et al.* (1987) found no impact on the fauna beyond 25m from the cages and Gowen *et al.* (1988) found no detectable effect beyond 30 m. In contrast, Weston (1990) detected an impact on selected physico-chemical parameters at a distance of 100 m, and Wu *et al.* (1994), under sub-tropical and significantly less technologically advanced growing conditions, found impacts over much greater distances (1-1.5km). The spatial patterns observed in both abundance and species composition of benthic infauna at the two salmon farms in our study are similar to those described by Pearson and Rosenberg (1978) in response to general organic enrichment sources and by Wildish *et al.* (2001) for aquaculture. However, although conditions under the cages were clearly impacted, there was no azoic zone, which was the worst case scenario described by Pearson and Rosenberg (1978). Similarly, no azoic zone was observed by Karakassis *et al.* (2000) in their study of finfish cage farming in the Mediterannean.

The multivariate analysis of the benthic community data also suggested a temporal change in levels of organic enrichment. This was particularly obvious at Nubeena where all sites next to cages were classified as moderate impact at the commencement of sampling, and progressed to major impact 10 months later. Although evaluation of seasonal variation in species abundances was not part of this research, the data indicate that recruitment occurred in some species over the course of this study. Recruitment of several species was particularly noticeable during the autumn sampling (5 month at Nubeena and 6 month at Hideaway Bay). This suggests that repeated environmental monitoring surveys should be conducted at the same time of year, and potential recruitment bias in the data should be considered for autumn samplings.

At both the Nubeena and Hideaway Bay farms, k-dominance curves generally identified the same highly impacted sites as multivariate analysis. However, the two analytical methods differed to a greater extent in the classification of unimpacted and minor impacted sites. At Nubeena, curves for sites determined by multivariate analysis to be moderately impacted (Group 2b) generally overlapped with those of the background unimpacted sites (Group 1), and these groupings could not be easily separated according to their k-dominance curves. Similarly, at Hideaway Bay, sites classified by multivariate analysis as representative of minor impact, had k-dominance curves which overlapped between minor and major impact, and the levels of impact were not easily

discerned. K-dominance curves also differed from multivariate analysis at Nubeena reference site R1. Moderate impact was indicated at this site in several k-dominance plots, primarily because of large numbers of the introduced gastropod *Maoricolpus roseus* during the 5 and 10 month sample visits. This resulted in a high cumulative dominance of the first ranked species at these times and placed it in a comparable position to the farm sites. Cluster analysis (Fig. 4.3) also identified the R1 site at both 5 and 10 months as different to the remaining boundary and reference stations. However, this difference in the community structure was not as great as between R1 and the farm cage sites. Thus, k-dominance plots and multivariate assessment can differ in the way they differentiate between sources of impact. Karakassis *et al.* (2000) also encountered inconsistencies in the assessment of conditions using multivariate techniques and the abundance-biomass comparison ABC method; a technique which incorporates both k-dominance and species biomass curves. Their results suggest that the multivariate techniques were more accurate.

K-dominance curves did provide a useful visual representation of changing conditions at sites with high organic loading. Sites next to cages at both farms showed a clear temporal progression of increasing impact. However, a number of problems were encountered in employing k-dominance curves to assess environmental impact. It was much more difficult to present large amounts of data on k-dominance plots than can be shown with multivariate analysis. Thus many k-dominance curves need to be drawn, compared with one dendogram/MDS plot showing the results of cluster analysis. K-dominance assessment also doesn't take into account species identity and can group sites which are dissimilar in their community composition. Thus, k-dominance plots do not account for the nature of the disturbance, and will group sites according to the level of disturbance regardless of the cause. There is also no reduction in taxonomic effort in producing k-dominance plots because species level identification is still required. For these reasons, multivariate analysis is generally preferred to k-dominance curves.

Of all the major invertebrate faunal groups examined at both Nubeena and Hideaway Bay, only the annelids showed major changes in abundance which were consistent with increased levels of organic enrichment. Annelid (polychaete) abundance and in particular number of *Capitella sp*. (MoV 2558) markedly increased at the cage sites, and was highly correlated. *Capitella capitata* (*Capitella sp*. (MoV 2558) has been found to be highly abundant in areas of organic enrichment in numerous other studies (e.g. Pearson and Rosenberg, 1978; Weston, 1990; Hargrave *et al.*, 1997). These results indicate that assessment of the number of polychaetes or *Capitella sp*. (MoV 2558) per m^2 will identify the impacted sites too much the same degree, as does multivariate assessment. An increase in *Capitella sp*. (MoV 2558) by a factor of 20 over the reference sites was indicative of a moderate impact, whereas an increase in abundance greater than 50 times corresponded with highly impacted conditions. *Capitella sp*. (MoV 2558) comprising more than 50% of the total faunal abundance was also only associated with highly impacted conditions directly under a cage.

Abundance of other invertebrate groups did not show any clear and consistent patterns between farms that could be related to the level of organic enrichment. Beneath the cages, reduction of the oxic zone would have reduced the amount of sediment that organisms can inhabit. Many of the common crustacea associated with soft sediments are burrowing or tube building and would be displaced under these conditions. Overall,

numbers of crustaceans per m^2 declined over time. However, at Nubeena densities at the farm sites were higher than at boundary and reference sites. This was probably a result of mobile epifaunal scavengers (e.g. *Nebalia* spp.) taking advantage of the increased food available at these sites. At Hideaway Bay the numbers were generally lower and very variable. Only the inshore and F 60 m groups at Hideaway Bay had significantly higher abundance levels, which may be due to the coarser sediment and patchy reef found at these sites. Additionally, crustacean densities increased at some cage sites at both farms due to biofouling of cages (e.g. *Caprella* spp.). Although these fouling species are linked to farming practices and may be useful as indicators of cage presence, they are not directly linked to the level of organic wastes from fish.

Abundance of echinoderms also varied between the site groups, and did not show patterns consistent with those identified by multivariate analysis. At Hideaway Bay echinoderms were completely absent from sites next to cages, however, they were also absent from some of the reference sites. Similarly, mollusc abundances at both Hideaway Bay and Nubeena farms did not show any of the patterns identified by multivariate analysis. The R1 reference site at Nubeena at the 5 and 10 month surveys was significantly different to most other sites because of the large increase in abundance of the introduced New Zealand screw shell, *Maoricolpus roseus*.

Abundances of molluscs, echinoderms and crustacea thus do not appear to be suitable indicators of organic enrichment for an environmental monitoring program. Nevertheless, the results suggest that if any of these major faunal groups are absent at a site but are present at reference sites, then further investigations should be conducted.

Number of species (species richness) is frequently used as a measure of environmental degradation, with a reduction in the number of species being characteristic of an environmental impact, particularly organic enrichment (e.g. Weston, 1990; Henderson and Ross, 1995). However the data obtained in this study do not clearly support this assumption. At the cage sites where major organic enrichment was indicated by multivariate analysis, annelid abundance and species composition, the number of species was not significantly less than at the reference sites. Instead, the number of species remained relatively stable at all sites and organic enrichment at the cage sites resulted more in replacement of species and increased abundance. At Hideaway Bay sites next to cages had reduced diversity compared to the boundary sites, but not in comparison with some reference sites. It is possible that the effects of organic enrichment observed in this study were not severe enough to result in a significant decrease in number of species. These results thus suggest that a decrease in the number of species should not be used in isolation as a determinant of environmental impact. However, because number of species has been found to be a useful indicator of organic impact in other studies (Pearson and Rosenberg, 1978; Weston, 1990; Horwitz and Blake, 1992) it would be prudent to still use species richness in conjunction with other indicators of organic enrichment to evaluate environmental conditions. Thus, a reduction in the number of species by 50% should be noted and taken into consideration in the overall evaluation. It is also important to note that subsequent to this study higher levels of impact, where the fauna decreased to very low levels or was absent, have been observed both in Tasmania and overseas (Pearson and Rosenberg, 1978; Rosenthal and Rangeley, 1988; Kupka-Hansen *et al.*, 1991; Holmer and Kristensen, 1992).

Number of individuals clearly distinguished the sites next to cages at the later surveys at both farms, i.e. at 10 months at Nubeena, and at 9 and 11 months at Hideaway Bay, and these were the times indicated by other analyses to be the most impacted by organic enrichment. Densities of individuals were much lower at Hideaway Bay than Nubeena at these times, however higher densities have been observed at Hideaway Bay in subsequent studies. The pattern of change in total abundance was very similar to that for annelid abundance and *Capitella sp*. (MoV 2558) abundances. From the results, an increase of approximately 10 times the mean total number of individuals recorded at the reference sites appeared to be indicative of moderate organic enrichment, and an increase of 20 times or greater indicated a highly impacted environment.

The Shannon Diversity Index clearly separated impacted cage sites from all others, and a Shannon Index value of less than 1 may be considered to be indicative of major disturbance. In this study, only sites next to cages fell below 1 and only after cages of fish had been in place for at least 9 months. Other sites identified as highly impacted by multivariate analysis at Hideaway Bay and moderate to highly impacted at Nubeena, had mean Shannon Diversity index values <2. At both Nubeena and Hideaway Bay, reference and boundary sites had high Inverse Simpson index values, and organically enriched communities next to cages and 10 m away showed low values. Generally, an Inverse Simpson index of <6 was indicative of moderate impact, and <2 of highly impacted conditions.

Multivariate and univariate assessment indicated that the same groups could still be identified at family level as were apparent at species level. This is consistent with the findings of Somerfield and Clarke (1995). With increasing taxonomic level above family, the moderately impacted sites became more difficult to distinguish. However, even at the highest levels, sites showing major impact were still easily distinguishable. This agrees with the suggestion made by Pearson and Rosenberg (1978) that the more severe an environmental impact, the higher the taxonomic level at which it will be evident. Thus the decision on the most appropriate taxonomic level depends on the size of effect to be detected. If it is sufficient to detect only the most severe impacts then evaluation to class or phylum level alone may be appropriate. However, the ecological information that can be gathered from assessment to this level is minimal and ultimately an equivalent evaluation may be obtained from a simpler sampling procedure such as redox measurement, or video evaluation.

The results from both farms indicate that it is important to analyse the benthic infaunal data in several ways to obtain an overall picture of the level of impact. This is because different analytical methods and indices can interpret the data differently, especially at low levels of impact. For example, the reference site R4 at Hideaway Bay was shown to be relatively unimpacted by multivariate analysis, but moderately impacted at the 3 and 9 month surveys by both the k-dominance curves and the Shannon Diversity index. This site also had the lowest mean number of species and lowest mean total number of individuals m^{-2}. R4 was the site furthermost out in the Huon River and had a very fine substrate with high organic matter content. This site may have been naturally depauperate or it may have been exhibiting an impact from other disturbances in the Huon River. Similarly F 0 m at Hideaway Bay was shown to be highly impacted by multivariate analysis, k-dominance curves, density of *Capitella sp*. (MoV 2558) and

Shannon Diversity index, but only moderately impacted by the Inverse Simpson index and total number of individuals.

The two farms investigated showed some obvious differences in benthic fauna, indicating differences in effects of salmon farm production levels and management practices on the environment, and/or different environmental conditions at each site. For example, the density of *Capitella sp*. (MoV 2558) was over an order of magnitude higher at Nubeena than at Hideaway Bay. Thus, although general categories for determining levels of impact have been identified, comparisons at each farm between sites near cages, compliance sites 35 m from lease boundaries and reference sites, are most important. Changes in fauna over time at each farm will also assist in the identification of level of impact.

The criteria developed at the two farms for identifying levels of impact represent a starting point. They will need to be regularly reviewed as more data become available from farms with different environmental conditions, salmon production levels and farm management practices. Subsequent research, for example, has shown that organic enrichment from salmon farms can have a greater impact on the environment than observed in these studies. In particular, intermittent periods of heavy organic enrichment conducted over several years has been observed to reduce the number of species and abundance of individuals to much lower levels than found in the present study.

The results of this study suggest that overall both multivariate and univariate analysis of faunal information at family level resulted in the loss of relatively little information over species level assessment. Identification at family level appeared to be sufficient to detect moderate impacts. A major criticism levelled at assessments conducted at higher taxonomic levels, such as phylum, is that these assessments produce little meaningful information on the nature of the response (Somerfield and Clarke, 1995). On the other hand, a criticism of species level studies is that incorrect identifications could give misleading information (Ellis, 1985). At family level the taxonomic information available about the multivariate groups is still sufficient to provide relevant ecological information. Identifying the fauna to family level will also help to alleviate the problem of incorrect identifications. Warwick (1988b) suggested that family groups could be readily recognised by ecologists with only moderate experience. Therefore, when cost for effort is considered, family level would appear to be an appropriate level. However, where species of particular scientific/ecological interest are present or other special circumstances exist, species level evaluation may be necessary.

4.5 Summary

The results of the macrofaunal analysis indicate that the composition and abundance of the benthic infauna is a sensitive and reliable method to assess the level of organic enrichment and disturbance associated with cage aquaculture practices. Benthic infauna can detect an effect up to 35 m from an operational cage system and a gradation of effect can be distinguished according to the distance from the cage and with the duration and intensity of culture.

The results also show that the benthic infaunal data should be analysed in several ways to determine the level of impact at a site. This includes using multivariate analyses, and several univariate indices of diversity. The ultimate categorisation of impact levels does not require that all indices are in the appropriate category, but that an overall indication of the level of degradation is obtained from various measures.

Some criteria for identifying levels of impact have been identified as follows. However, it must be emphasised that these are preliminary as they are based on only two farms. These criteria will need to be regularly reviewed as more data from different environments and different levels of salmon production become available.

- MDS plots - Impacted sites are distinguished by species composition and low similarity with reference sites. Any site which lies within the 90% confidence kernels of an impacted cluster grouping would also be impacted.

- K-dominance curves - Moderate impact is indicated when the first ranked species comprises approximately 30-60% of the cumulative dominance. Greater than 60% cumulative dominance of the first species, and quickly reaching 100% is indicative of a major impacted site, and >90% dominance of the first species a severe impact. Also, if the k-dominance curve for a site lies above a curve for sites next to cages, then a high impact is indicated.

- Number of Species - A reduction in the number of species by 50% should be noted and taken into consideration in the overall evaluation. A defaunate sample is an indicator of high impact.

- Number m^{-2} - An increase of x10 indicates moderate impact, and an increase of x20 (or a defaunated community) implies that a high level of impact has occurred.

- Shannon Diversity Index - A Shannon index of less than 2 indicates moderate impact, and a Shannon index of less than 1 indicates high impact.

- Inverse Simpson Index - An index value less than 6 indicates a moderate environmental impact has occurred. An index value less than 2 implies a high level of impact.

- Species composition - *Capitella sp*. (MoV 2558) comprising more than 50% of the total number of individuals indicates at least a moderately impacted site. A defaunate site would be indicative of severe impact.

5. Number Of Benthic Infaunal Samples Required To Reliably Assess Environmental Impact

5.1 Introduction

The Department of Primary Industries, Water and Environment (DPIWE) and the Tasmanian Salmon Growers Association (TSGA) debated for some time about the requirements for environmental monitoring of salmon farms. There was considerable discussion about which environmental variables should be monitored, how often and how many samples should be taken at each farm. Industry was also concerned about the costs that would be incurred in conducting an intensive monitoring program. An agreement was reached between industry and DPIWE on the environmental variables to be measured for the baseline environmental assessment of marine farms before they commenced operation. Requirements for ongoing environmental monitoring were also agreed to in principle, but several issues still remained to be resolved. One of these issues related to monitoring the benthic infauna - how many samples should be taken and how often?

Research conducted in several countries overseas and by independent scientists has concluded that the composition of the benthic infauna is one of the best indicators of environmental change, especially at low levels of impact (e.g. Cochrane and Pearson, 1995; GESAMP, 1996; Pohle and Frost, 1997). The baseline environmental monitoring program for salmon farms, which was agreed to by DPIWE and industry, included taking samples of the benthos at several sites around the farm. For a hypothetical 20 ha farm shown below (Fig. 5.1) benthic samples were collected using grabs or cores at the sites marked with an X. These included four sites 35 m from the boundary of the farm, two sites within the farm, and at a Control (reference) site. The 35 m mark from a farm boundary was chosen because any environmental effects from a cage located at the boundary were unlikely to extend this far, based on overseas research. It was agreed that only one Control site would be required if the farm site was homogeneous in substrate characteristics and the one Control site was representative of the conditions on the farm. At that time, DPIWE was requesting that triplicate benthic samples were collected at each site. Thus a minimum total of 21 benthic samples would require sorting and identifying.

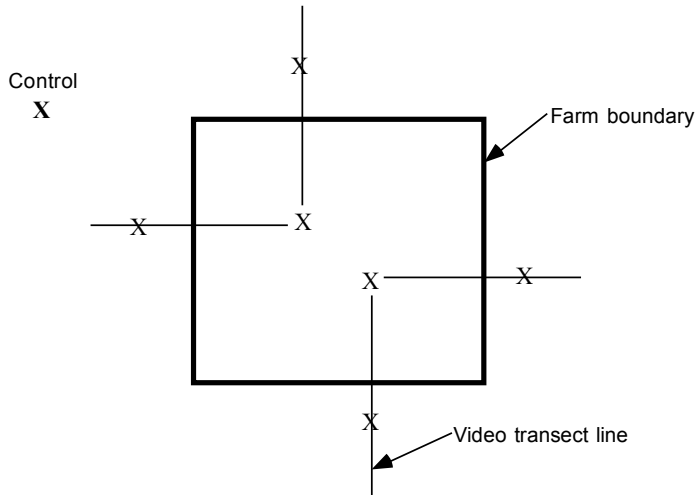


Fig. 5.1. Location of sample sites, denoted by X, at a hypothetical 20 ha farm (not to scale).

Assessment of the benthic community has been found to be laborious and requiring considerable expertise that can not be readily acquired (e.g. Warwick, 1988b). Hence it is a comparatively expensive environmental variable to measure. Rumours were circulating amongst the Tasmanian aquaculture industry of costs of $300 - 400 per site for benthic invertebrate assessments, but quotes from one local company were $100 per sample for benthic sorting and identification. The question raised by industry was whether three replicates at each site were necessary because this incurred considerable costs to the farmer. Industry representatives proposed that only one sample was required to give an indication of the conditions at that site, and that if the one sample showed evidence for a change in environmental conditions, then more samples should be collected and analysed.

At a meeting between DPIWE and Tasmanian salmon growers on 30 July 1997 there was considerable debate over sample size requirements, and industry representatives requested that an assessment be conducted of the number of replicates of benthic fauna that would be required for environmental monitoring of salmon farms. This project was developed in response to industry's request.

5.1.1 Objectives of environmental monitoring

Both industry and DPIWE have agreed that environmental monitoring of salmon farms is necessary in order to detect if any change is occurring due to marine farming activities, and that it must be conducted in the most cost effective way possible. However, the two groups expressed some differences of opinion in how the monitoring program should be conducted and the information they would like to receive.

DPIWE's main objective for environmental monitoring was to meet legislative requirements that any adverse impacts of marine farming were minimal and that no unacceptable environmental degradation occurred outside the lease area. The Management Controls in the Marine Farming Development Plans for each growing area stipulate that:

> "There must be no unacceptable environmental impact 35 m outside the boundary of the marine farming lease area. Relevant environmental parameters must be monitored in the lease area, 35 m from the boundary of the marine farm lease area and at any control site(s) in accordance with the requirements specified in the relevant marine farming licence".

Industry also recognised that it was important to monitor the effects of marine farms on the environment so that the farmers, government authorities and the general public know the extent of impact of farming on the environment. At the same time they also want to collect data that are useful for their own farm management purposes, such as when to fallow. Thus an additional objective for industry was to collect data related to farming activities, in particular, effects on the environment in high impact areas such as under cages and at fallowing sites. From these data they could develop better farm management protocols.

Although taking replicate samples is more costly, there are many benefits. Variation in the benthic invertebrate community between sampling sites due to natural causes will occur, and if only one sample is taken then the chance of detecting a large difference between sites is much higher than if several samples are averaged. Thus by taking replicate samples there is less chance of falsely detecting pollution effects and having to take additional samples.

Replicate samples provide a measure of the natural or chance variability that is occurring at a site. Such measures of variability then serve as a yardstick to determine if observed differences between sites or observed differences over time are large enough to be a real difference, or are simply due to chance or natural variability.

5.1.2 Objectives for determining the number of samples required

The assessment of sample size requirements for benthic fauna thus needed to consider two objectives:

1. Sample size required to detect unacceptable impact at 35 m from the salmon farm boundary.

2. Sample size required to provide information relevant to the development of farm management practises, in particular when cages should be moved because the environment under the cage has deteriorated to an unacceptable level, how long cage sites should be fallowed, and at what level of environmental recovery cages can be restocked.

This chapter largely relates to Objective 1. However, monitoring at the farm boundaries does provide a potential warning system for farmers of environmental health within the farm.

5.2 Methods

5.2.1 Analysis of environmental data to detect impacts

The standard method used worldwide for the assessment of human induced environmental impact is the BACI (Before, After, Control, Impact) design (Green, 1989). This requires environmental variables to be measured at control and impact sites both before and after the impact activity occurs. Comparisons are made of environmental variables before to after impact, and between control and impact sites. Significant changes in environmental conditions are tested using the statistical procedure Analysis of Variance (ANOVA). In recent years the design of environmental monitoring programs and analysis of impacts has become more powerful. This includes measurements of environmental parameters at several times before and after the impact activity occurs to provide greater information on temporal variability, and at multiple Control sites (and impact sites if possible) to detect a greater variety of impacts (see chapters in the book by Schmitt and Osenberg, 1996 on 'Detecting Ecological Impacts').

The recently developed analytical methods provide a greater reliability of detecting whether an impact has, or has not, occurred which is of benefit to both the regulator and the operator. False detection of impacts can cause considerable unnecessary costs to the operator because he may be required to conduct more environmental assessments and change their operations when in fact there hasn't been an impact. Whereas failing to detect an impact when it has in fact occurred can lead to continued significant environmental damage.

The BACI type design is used as the basis for analysis of environmental impacts of salmon farms, and hence the number of samples required.

5.2.2 Strategy for analysis of environmental monitoring data from salmon farms

The assessment of the number of samples required from salmon farms in Tasmania was based on detecting an impact at 35 m beyond the farm boundaries. The design has been developed from the following farm layout (Fig. 5.2) which is slightly different to that described for the baseline assessment in Fig. 5.1, and includes an additional source of variation which does affect the number of samples required, as will become evident in the power analysis.

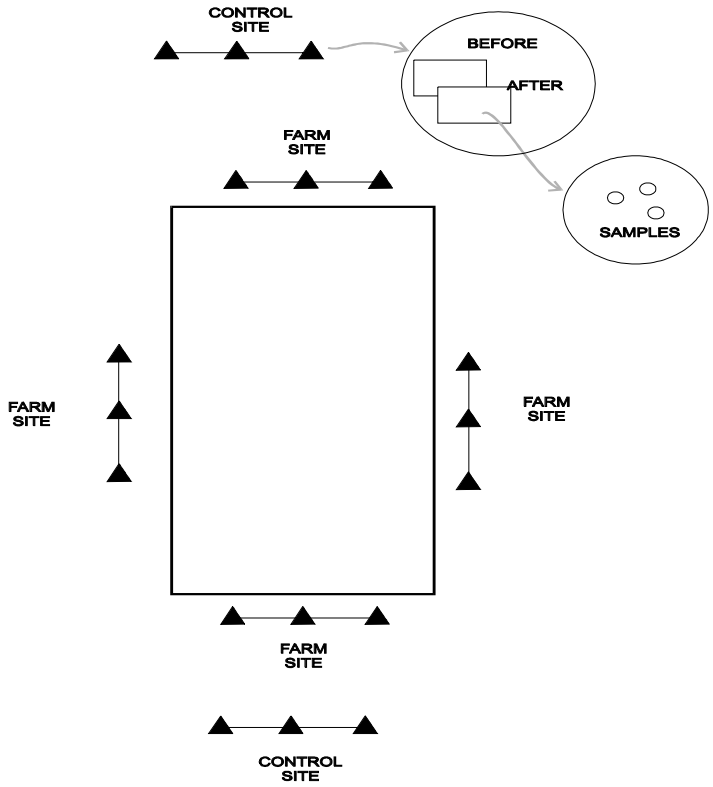


Fig. 5.2. Proposed positioning of sites, and structure for within sites positioning of sample points, samples and the first two time-based measurements (not to scale). Farm sites are 35 m outside the farm boundary. Note: the number of sample points per site and number of samples per point is not necessarily three.

A standard farm is monitored at 35 m outside each boundary, i.e. monitoring occurs at four farm sites as shown in Fig. 5.1. Each site consists of a transect with sampling points identified at two or more points along the transect. At each point of sampling, there is to be one or more samples obtained. The recommended number of farm sites, sampling points and samples depends on the natural variability occurring between sites, between sampling points and between samples and is estimated from the analysis of the variance of each component.

The importance of multiple sampling points along the farm sides is dependent on the manner in which organic material moves away from the farm. If it moves in a broad band, then a single sampling point could detect it. If, however, the organic matter moves away in a small ribbon, then more sampling points are necessary to detect an impact.

There is also a temporal component. Data are to be collected at fixed points in time from every sampling point. From the initial two samplings, a study of the difference is to be made. As data are obtained over a longer period, the analysis can be used to seek evidence of longer term trends.

5.2.3 Sources of variation

There are three components of unexplained (or natural) variation that contribute to sampling variation in the site means. These are:

1. *Between site* variation which reflects unexplained (i.e. non pollution) sources of variation between sites.

2. *Between sampling point* variation which represents the unexplained variation that occurs among positions along the sides of the farms.

3. *Between sample* variation which is the unexplained variation between samples collected from a common sampling point.

Additionally, there may be variation due to pollution (organic enrichment). That variation may be exhibited as:

1. Differences among farm sites due to differing levels of pollution extending out at different points from the farm; and

2. Differences between levels at farm sites and control sites.

These sources of variation contribute to the comparison of mean values computed from different sites as follows:

Let n = number of sampling points per site, r = number of samples obtained at each point; VC_s, VC_n, and VC_r are the variance components from variation among sites, points within a site, and samples at a point, respectively, and P_1 and P_2 are contributions from pollution at Site 1 and Site 2, respectively. Then the expected variation in the difference between the observed means at Site 1 and Site 2 is

$$V_b = (P_1-P_2)^2 + 2(VC_s + VC_n/n + VC_r/nr) \qquad \textit{Between farm site variation}$$

The expected variation among means recorded at each point at a single site, i.e. the 'within site' variation is

$$var = VC_n + VC_r/r \qquad \textit{Within site variation}$$

If the expected within site variation is multiplied by $2/n$, then it matches the last two terms of the Between site variation. It is apparent that the Between site variation will be larger than the within site variance if either of the two following conditions apply:

(i) there is a difference in organic matter levels at the two sites, or

(ii) there is natural site-to-site variation.

In summary, if Analysis of Variance is employed using the *within* site variation as a yardstick to check for evidence of organic enrichment*, there is the likelihood that spurious claims of pollution will be made*. What is claimed to be evidence of organic enrichment may be natural between site variation.

The only effective yardstick for seeking evidence of pollution is the between control site variation. The variation of the difference between two control sites is:

$V_c = 2(VC_s + VC_n/n + VC_r/nr)$ *Between control site variation*

5.2.4 Importance of measuring control sites

1. They provide protection for the fish farmers against false claims of environmental impact. In the absence of control sites, any increase in organic material would be attributed to pollution being discharged from farms. If the pollution were coming from other sources, this fact would not be registered, and the fish farm would be wrongly accused of being the polluter. By using changes at control sites as the yardstick, fish farms will be the alleged polluters only where there is real evidence supporting the claim.

2. The variation between control sites is the only variation which provides a measure of the variability due to non-pollutant sources. It is the variation among control sites which provides the yardstick for formally assessing if changes over time at a site, or differences among sites are related to pollution or are simply natural variation.

5.2.5 Manner in which organic matter spreads

The process of detecting changes due to organic enrichment is complicated by the fact that this may not spread evenly from all sides of the farm. This seriously affects the choice of best form of statistical analysis. The two extremes and their detection are as follows:

1. Uniform spread of pollution. In this case, the best test compares the mean of all farm sites with the mean of the control sites, with the expectation being that the mean of the farm sites would be higher if there is pollution.

2. Uneven spread of pollution. If the pollution was confined to one side of the farm, then only one of the four site means would be affected. To detect this situation, the mean for each farm site can be compared with the control mean.

5.2.6 Strategy for checking for pollution

The following steps form the basis of a test for evidence of change in pollution levels over a period of time, where measurements are recorded at the same points at the beginning and end of the time period. The following steps use data formed as the differences, i.e. ‘after-before’ in the pollution indicators.

Step 1. *Is there evidence of change at any farm site?* Test the hypothesis that there has been no change at a farm site. This should be done individually for each farm site on the grounds that pollution may affect the different monitoring sites differentially.

If there is no evidence of change at any farm site, then either declare that there is no evidence of pollution and make no further statistical tests at this time, or go to Step 1(a).

If there is evidence of change at one or more farm sites, then go to Step 2.

Step 1(a). *Is there evidence that the mean for farm sites shows a change?* Test for evidence that the mean level across all farm sites has not changed. This is a more powerful test than are tests on individual farm sites if the pollution is uniformly discharging across boundaries. It is only recommended if a uniform spread of pollution is seen as a reasonable assumption.

If no evidence of change is detected, then declare that there is no evidence of pollution and make no further statistical tests at this time.

If there is evidence of change, go to Step 2(a).

Note: The evidence for change at farm sites is calculated using the estimate of natural variation among the control sites. The variance between the Controls is included in the calculations so that only the change above natural variability will be detected. Without this Control variance, the farmer could be accused of impacting on the environment, when in fact the change was only due to natural variability.

Step 2. *Is there evidence that the change at any farm site is greater than the change at control sites?* Test the hypothesis that the change at a farm site is no greater than the change which is recorded at control sites.

If this hypothesis is accepted, then declare that the increased pollution level recorded at the farm site is explainable as being due to sources external to the farm.

If the hypothesis is rejected, then conclude that there is evidence of pollution at the farm site which is due to pollution from the farm. The likely size of the increase can be provided using a confidence interval.

Step 2(a) *Is there evidence that the mean for farm sites shows a change which is greater than that shown at control sites?* Test for evidence that the change in mean level across all farm sites is no greater than that observed at control sites.

If this hypothesis is accepted, then declare that the increased pollution level recorded at the farm sites is explainable as being due to sources external to the farm.

If the hypothesis is rejected, then conclude that there is evidence of pollution at the farm sites which is due to pollution from the farm. The likely size of the increase can be provided using a confidence interval.

5.3 Computational Details

5.3.1 Preliminary calculations

1. Compute the changes in means (i.e. after-before) for the m control sites. Call these $c_1, c_2, \ldots, c_m$ and compute the overall mean change for the control sites, $c = (c_1 + c_2 + \ldots + c_m)/m$.

2. Compute the variance for the set of control means. Call this s^2.

3. Compute the changes in means for the n farm sites. Call these $f_1, f_2, \ldots, f_n$, and compute the overall mean change for the farm sites, $f = (f_1 + f_2 + \ldots + f_n)/n$.

5.3.2 Tests

Step 1. *Is there evidence of change at any farm site?* Using the mean for each farm site in turn, compute values of the statistic $t_i = f_i/s$ using values of $f_1, f_2, \ldots, f_m$. Use the t-distribution tables with degrees of freedom equal to number of control sites minus one, i.e. m-1, to determine the value $t_{0.05}$ which satisfies Pr ($t > t_{0.05}$) = 0.05. If the observed value for any site exceeds $t_{0.05}$, then there is evidence of increasing pollution at that farm site.

Step 1(a). *Is there evidence that the mean for farm sites shows a change?* Compute a value of the statistic $t = f/(s/\sqrt{n})$. Compare the value of t with $t_{0.05}$ obtained in Step 1. If the computed value of t exceeds $t_{0.05}$ there is evidence that the average level of pollution around the farm sites has increased.

Step 2. *Is there evidence that the change at any farm site is greater than the change at control sites?* Using the mean for each farm site in turn, compute values of the statistic $t_i = (f_i - c)/(s\sqrt{[1+1/m]})$ using values of $f_1, f_2, \ldots, f_m$. If the observed value for any site exceeds $t_{0.05}$, then there is evidence that increasing pollution at one of the farm sites is greater than that at the control sites.

Step 2(a). *Is there evidence that the mean for farm sites shows a change which is greater than that shown at control sites?* Compute a value of the statistic $t = (f-c)/(s\sqrt{[1/n+1/m]})$. Compare the value of t with $t_{0.05}$ obtained in Step 1. If the computed value of t exceeds $t_{0.05}$ there is evidence that the average level of pollution around the farm sites has increased.

5.4 Deciding On The Number Of Sites, Sampling Points Per Site, And Number Of Samples Per Sampling Point - Power Analysis

Analysis of sample size for environmental monitoring programs which use ANOVA to test for significant environmental change, involves the statistical procedure called Power Analysis. Fairweather (1991) provides a simple explanation of the need for statistical power analysis in environmental biomonitoring.

Power analysis requires several types of information:

a the significance level set for a test, normally a = 0.05. This is the probability of statistical testing incorrectly concluding that an impact has occurred, also described as the probability of Type I error.

b the probability of statistical testing failing to detect an impact, also described as the probability of Type II error. Note: 1- b is called the power of the test and is the probability of correctly detecting that an impact has occurred.

δ the effect size, which is the magnitude of the minimum change (impact) to be detected.

s^2 the variability in the parameter being sampled.

n the sample size.

a is commonly set at 0.05. A commonly accepted value of power is 0.8 so b = 0.2. The variability of the data, s^2, can be determined from pilot data by calculating the variance. This provides an estimate of the natural variability in the measured environmental variables at each site. The effect size, δ, is the smallest pollution effect we wish to detect.

5.4.1 Power analysis for salmon environmental monitoring

Power analysis is employed to find the cheapest way to detect pollution above the minimum prescribed level under the conditions which have been imposed. The analysis of number of samples required is based on samples being taken at four farm sites (i.e. one on each boundary) for the initial implementation of the monitoring program. Variation is assumed possible in the number of control sites (c), the number of sampling points per farm site, i.e. along each boundary (n) and the number of samples per sampling point (r).

Three factors determine the values of c, n and r. They are:

- *the capacity of the monitoring system to detect impact levels which are regarded as significant*
- *the inclusion of sufficient sampling points along each boundary to provide adequate detection of restricted pollution discharges; and*
- *the relative costs of different combinations that achieve the required power and the total amount of money available for the monitoring process.*

Statistics can contribute to choosing an optimal combination of numbers of control sites, sampling points and samples by determining the power of detecting minimum differences of practical significance between the mean change in farm site levels and the mean change in control levels at a range of combinations of c, n and r.

The calculations depend on availability of estimates of variance components and the minimum increase in pollution levels which are to be detected.

5.4.2 Variance components

Table 5.1 and Table 5.2 supply the best estimates of the relative size of variation from the three identified sources. This information is derived from analysis of data collected at Hideaway Bay by the marine environmental research group at the Marine Research Laboratories at Taroona as part of their research on determining the best methods to monitor environmental impacts of salmon farms. They surveyed the benthic invertebrate fauna at a number of sites around the farm, at three points along transects at each site, and three samples at each point. The farm sites were hundreds of metres apart, the sampling points were 15 - 45 m apart, and the samples were theoretically at the same point. Samples collected by diving were within 1 m accuracy, whereas grab samples were estimated to be within 5 m accuracy. The variance component results clearly show that for both total species and total abundance data, the greatest variation is occurring between samples taken at the same spot (57% contribution), compared with 30 - 38% contribution from samples taken at points along a transect, and 4 - 12% from samples taken at different farm sites.

Table 5.1. Variance component data (log) for 'total species'. B1 – B6 are boundary transects described in Chapter 2. B7 – B9 are additional boundary transects.

Source	B1-B2	B3-B6	B7-B9	Average	Percent contribution
Between farm sites	0.0000	0.0107	0.0000	0.0036	4
Between points on transect	0.0519	0.0000	0.0000	0.0173	38
Within sampling points	0.0311	0.0333	0.0070	0.0238	57

Table 5.2. Variance component data (log) for 'total number of individuals'. B1 – B6 are boundary transects described in Chapter 2. B7 – B9 are additional boundary transects.

Source	B1-B2	B3-B6	B7-B9	Average	Percent contribution
Between farm sites	0.0000	0.0235	0.0026	0.0087	12
Between points on transect	0.0612	0.0000	0.0013	0.0208	30
Within sampling points	0.0471	0.0502	0.0217	0.0397	57

5.4.3 'Effect size'

The effect size, δ, is the smallest difference we wish to detect between population means. It is anticipated that there will be some impact from the salmon farm within the lease area, but not at 35 m or more from the farm boundary. A severe detrimental impact on the benthic fauna generally results in three changes - (i) a dominance of pollution tolerant species (e.g. capitellids) resulting in a significantly increased total abundance, (ii) a decrease in the total number of species, and (iii) a change in community structure. Changes in community structure as a result of an environmental impact are best quantified in a single parameter suitable for power analysis by

investigating effect size in relation to total abundance per grab or per m^2, and total number of species per sample. If the impact worsens it can result in the loss of all species at the site, which would be evident from the total number of species present.

The management controls stipulate that the environmental impact must not be unacceptable at the 35 m point from the boundary, and the null hypothesis for the monitoring program will be that there is no significant difference in species number or total abundance between controls and sites 35 m from the boundary. Power analysis is being used to determine the number of samples that will be required to reliably detect a minimum difference (effect size) between controls and farm sites. We want to be confident that we can detect an effect size of a fourfold increase in total abundance and a twofold decrease in total species number. We have set the level of power at 0.8, meaning that if changes do exceed these limits the statistical analyses will detect this change in 4 out of 5 cases. These changes of a fourfold increase in abundance and twofold decrease in species number are substantial given that many members of the general public believe that environmental monitoring is to ensure that salmon farms have no impact outside the lease area. If significant change is detected then sampling at more sites would be required.

5.4.4 Power calculations

Suppose n is the number of sampling points per site and r is the number of samples obtained at each point; VCs, VCn, and VCr are the variance components from variation among sites, points within a site, and samples at a point, respectively. Then the variance of a site mean is

$$\text{variance} = VC_s + VC_n/n + VC_r/nr \qquad \textit{Site variance}$$

Power calculations for four situations are shown below. For use with all calculations, it is necessary to determine the value t_α which satisfies Pr ($t > t_\alpha$) = α from the t distribution with degrees of freedom n-1.

In the formulae below, s^2 = the estimated site variance, s = *standard deviation*, n = number of sampling points per site, r = number of samples taken at each point, m = number of control sites and t = number of farm sites.

1. *Detecting an increase in pollution levels at a farm site.* To detect an increase in the pollution level of δ, the power of the test is equal to Pr (t>[st_α-δ]/s) where t is assumed to have a t (m-1) distribution.

2. *Detecting an increase in mean pollution levels across farm sites.* Compute the standard error of the mean, $s_m = s/\sqrt{t}$, where t is the number of farm sites. To detect an increase in the pollution level of δ, the power of the test is equal to Pr (t>[$s_m t_\alpha$-δ]/s_m) where t is assumed to have a t (m-1) distribution.

3. *Detecting that an increase in pollution levels is greater at a farm site than at the control sites.* Compute the standard error of the difference between the site mean and the mean of all control sites, $s_d = s/\sqrt{(1+1/m)}$, where m is the number of control sites. To detect an increase in the pollution level of δ, the power of

the test is equal to Pr (t>[$s_d t_\alpha$-δ]/s_d) where t is assumed to have a t(m-1) distribution.

4. *Detecting that an increase in mean pollution levels at farm sites is greater than at the control sites.* Compute the standard error of the difference between the site mean and the mean of all control sites, $s_d = s/\sqrt{(1/t+1/m)}$, where m is the number of control sites. To detect an increase in the pollution level of δ, the power of the test is equal to Pr (t>[$s_d t_\alpha$-δ]/s_d) where t is assumed to have a t (m-1) distribution.

An Excel spreadsheet with the necessary power calculations was developed. Values of the relevant input variables (number of control sites, number of farm sites, number of sampling points and number of samples) were varied to explore different combinations. Variance estimates were taken from the information in Table 5.1 and Table 5.2.

5.5 Results Of Power Analysis For Detection Of Impacts Outside Farm Boundaries

5.5.1 Excel spreadsheets of power calculations

The Excel spreadsheet calculated the probability of detecting increased pollution for different sample sizes. Examples of this power analysis are presented in Appendix 1. For each set of values for number of control sites and number of farm sites there are four sets of results: (i) power to detect increased pollution at a single farm site, (ii) power to detect increased pollution averaged across farm sites, (iii) power to detect increased pollution at a farm site compared with control sites, and (iv) power to detect increased pollution at farm sites compared with control sites, each of which is presented on a separate page. The variance components used in the calculations and shown in the top left-hand corner are the average values from Hideaway Bay which are presented in Table 5.1 and Table 5.2. The number of farms and the number of control sites are specified below the variance components.

The tables in the lower half of each page list the combinations of *number of points per site* and *number of samples per point*, and the *standard deviation (sd)* which is a measure of the variation and depends on the number of samples taken. The *cut-off point* relates to the Type 1 error. If the cut-off point is raised, the chance of detecting pollution decreases when pollution is present, as does the chance of declaring that a change has occurred when it hasn't. The *size of difference* row lists different effect sizes, i.e. the minimum differences we wish to detect. For both total species and total abundance we have log transformed the data so we can investigate multiplicative effects: changes in total abundance or number of species by ½ - 2 x (log 2 = 0.301), 1/3 - 3 x (log 3 = 0 .477), ¼ - 4 x (log 4 = 0.6021) and 1/5 – 5 x (log 5 = 0.699). The columns under these headings list the power of detecting increased pollution. The minimum acceptable value of power is 0.8 or 80%.

5.5.2 Results of power calculations analysis

Results of power for total abundance of benthic infauna data for 4 control sites - 4 farm sites, and for total number of species of benthic infauna with 4 control sites - 4 farm sites are shown in Appendix 1. Appendix 2 calculates the total number of samples that would be required for different combinations of points per site, samples per point and numbers of control sites.

For total abundance data at 4 farm sites and 3 controls, the power to detect increased pollution of 1/5 to 5 x abundance at a single farm site only reaches 80% with 4 points per site and 3 samples per site, i.e. 84 samples in total (Appendix 2). Across farm sites greater than 80% power of detecting a decline in abundance by 1/3, or an increase by 3 x, is best achieved by having three points along each transect and one sample per point. i.e. 21 samples in total. With 3 control sites the power to detect increased pollution at a farm site compared with control sites never reaches 80% at the effect sizes listed (> 160 samples), and across all farm sites compared to controls a 1/4 decline or 4 x increase in abundance reaches acceptable power at 3 points per site and two samples per point (42 samples in total).

If the number of control sites are increased to 4 (Appendix 1a), the power to detect increased pollution at a single farm site approaches acceptable levels for a 5 x increase in abundance at 3 points per site and 1 sample per point, i.e. 24 samples in total. Across all farm sites, 1/3 to 3 x change in abundance is best detected at 2 points per site and one sample per point (16 samples in total). Increased organic enrichment at a farm site compared with controls is detected at 80% power at 5 x increase in abundance at 3 points per site and 2 samples per point (total of 48 samples), but across all farm sites compared with controls, acceptable power for a 4 x increase in abundance is best detected using 2 points per site and one sample per point (16 samples in total). Thus, by increasing the number of control sites by one, the power to detect pollution reaches the acceptable 80% level with less number of total samples required.

Similar results are obtained using total species data. However, the number of samples required to detect a twofold decrease in species number with four control sites was high (24 - > 160). With 4 farm sites and 4 control sites (Appendix 1b), a change in number of species by 1/4 or 4 x is best detected at the accepted level of power at a single farm site, and at a farm site compared with controls, from the combination of 3 points per site and one sample per point (24 samples).

5.5.3 Recommended sample requirements

Analysis of the number of samples required using power analysis with 4 farm sites and various combinations of the number of control sites, points per site, and samples per point, and taking the costs of analysis into consideration, indicates that acceptable power of detecting increased pollution is achieved with 4 control sites, 3 points per site and one sample per point, 24 samples in total. Thus instead of taking replicate samples at the one spot, samples are required to be taken 20 - 30 m apart on a transect 35 m outside the farm boundary. Samples over time would be required from these points 20 m apart, which could easily be relocated using DPGS equipment.

This combination of sample numbers is also appropriate if the number of farm sites is reduced to 2 (one upstream and one downstream), after it is shown that there is a strong directional current flow which spreads the wastes from the farm in the direction of the current.

This total number of 24 samples to monitor the impact of the farm on the environment is 3 more than was proposed for the baseline assessment of salmon farms. This is because 3 additional control sites are required. The usefulness and reliability of the results obtained, however, is so much greater, that it would be better to take more samples less often, than a few samples regularly.

The number of samples required also should be reviewed annually, particularly as more data become available. By having a dynamic process with regular reviews and upgrading, the best monitoring system both in terms of scientific value and value for money can be achieved.

5.6 Summary

- Changes to the benthic infauna are widely recognised as one of the best indicators of environmental impact from fish farms, but benthic faunal composition is relatively expensive to analyse. This analysis has been conducted to determine the minimum number of samples that would be required for a monitoring program to reliably assess the state of the benthic environment around salmon farms.

- The number of samples was assessed in relation to three levels of sampling: number of sites at each farm, number of sampling points along a transect at each farm site, and number of samples to be taken at each sampling point. Variation between samples taken at each level was calculated from data collected at Hideaway Bay in the Huon Estuary.

- Power analysis was used to determine the power of detecting increased organic enrichment at various combinations of number of control sites, number of sampling points at each farm site and number of samples at each sampling point. The number of farm sites was initially set at four, one at each boundary. Power analysis also required the magnitude of the change to be detected, and this was set at a fourfold increase in total abundance, or a twofold decrease in total number of species. However, the number of samples required to detect a twofold change in species number was considered to be impractically high, and as a trade-off this was increased to a fourfold change.

- From the power analysis the best combination of sampling at 4 farm sites to be able to detect an increase in pollution 35 m from the farm boundary at an acceptable level of power was found to be:

  4 control sites, 3 sampling points and 1 sample at each sampling point, i.e. single samples to be taken approximately 20 m apart at 3 points on each of 4 farm boundaries and at each of four control sites, a total of 24 samples.

This analysis of number of samples required shows that more control sites are required than originally planned. By using more control sites the same level of accuracy can be

obtained with fewer overall samples. However, if compromise is necessary then it is recommended that benthic infaunal analysis is conducted less often but with the required number of samples for accurate analysis, rather than fewer samples taken more frequently.

- It is important to recognise the benefits of taking additional control samples. Controls provide information on the natural variability between samples, and whether an environmental impact is coming from the farm or from other sources. Without this control information, industry can expect to regularly receive false claims of pollution, which will require additional sampling and changes to their farms at substantial costs.

6. Video Assessment Of Environmental Impacts Of Salmon Farms

6.1 Introduction

Video recordings of the marine environment are increasingly being included in environmental monitoring programs for a variety of reasons. In recent years video equipment has improved in quality but decreased in costs. Video records of marine environmental conditions are easy to collect compared to measuring many other environmental parameters. They are also relatively inexpensive and provide an instant record of conditions on the bottom which can be easily viewed and interpreted by all interested parties. Finally, they provide a permanent record which can be readily stored and retrieved at a later date for comparisons over time.

Video records are now being used routinely to assess impacts of fish farms in many countries, and are considered to be a valuable monitoring tool because they provide evidence of changes that occur as a result of farming activities, e.g. Scotland (SEPA, n.d.), Maine USA (Heinig, 1996), New Brunswick (Chang and Thonney, 1993) and British Columbia (British Columbia Environmental Assessment Office, 1998). They are, however, subject to individual interpretation and some training and experience is required in their use (Heinig, 1996).

There is little published information on the suitability of video assessments for monitoring environmental impacts of fish farms compared with other monitoring methods such as changes to benthic infauna or physical parameters. Also, most assessments of video recordings have involved generalised descriptions of the benthic environment or detailed written descriptions (e.g. Krost *et al.*, 1994). Such descriptions are subjective, can not be analysed quantitatively, are time consuming and it is difficult to detect changes over time. In a comparison of variables used for environmental monitoring of aquaculture, GESAMP (1996) lists visual surveys of large invertebrates and demersal fishes by still photographs or videotape as being frequently used, but of low cost and low interpretative value because observations are typically only qualitative. Cheshire *et al.*. (1996) found that video surveys were useful for monitoring seacage farming of tuna, but they recommended that refinement of techniques was required for routine monitoring. Generally temporal comparisons of video recordings require replaying to review previously recorded information. A pictorial presentation of key video observations which enables visual comparisons of changes over time was developed by Heinig (1996) for salmon farms in Maine USA, but does not permit quantitative assessment.

The objectives of this component of the research were twofold. Firstly, they were to develop applicable techniques for video monitoring and to develop a quantitative assessment of video recordings that would be relatively simple and quick to conduct, and appropriate for a long term monitoring program. Secondly, they were to assess the suitability of benthic visual information recorded on video compared with other environmental variables, such as the benthic infauna, for monitoring the environment around marine farms.

6.2 Methods

Video recordings were made along transects at both salmon farms. The 60 m boundary transects (described in Chapter 2) were filmed, and triplicate samples for investigation of benthic infaunal community structure were collected at the 0, 45 m and 60 m points along the transect (the 45 m site was at the 35 m compliance point from the boundary). Reference transects (R's) 25 m in length were also recorded on video and triplicate sediment samples were collected from one end. Transects next to operational cages were also assessed at both farms. At Nubeena a long farm transect, extending around 3 cages to 35 m beyond the third cage, was videoed, and triplicate sediment samples were collected at the edges of the cages, F1, F2, F3, and 35 m from the cage edge, F35. At Hideaway Bay the farm transect (F) extended from the edge of a stocked cage to 60 m away. However, this transect was difficult to video using the ROV because of the arrangement of moorings and predator netting, and only the video footage of the first 10 m from the cage at all sampling times, and F 10-20 at the 6 month sampling visit, were of an acceptable standard for analysis. Triplicate sediment samples were collected at the edge of the cage, and at distances of 10 m, 35 m, and 60 m.

DGPS co-ordinates were used to deploy transect lines (marked every 5 m) before filming and to locate sampling stations. In shallow water < 20 m, a diver operated Hi-8 underwater video camera was used (Blaupunkt Video Camera Recorder Model CC984 (Hi-8 Pal) 10x zoom colour camera). In deep water at Hideaway Bay, a private company was hired to film the bottom using a Hydrovision Hyball Remote Operating Vehicle (ROV). Transect lines, marked every 5 m, were deployed using DGPS at the surface before each filming, with the exception of the long transect around fish cages at Nubeena where only the last 35 m extending out from the cages was videoed using a transect line.

Preliminary research included developing appropriate procedures to ensure that the video footage obtained was of high enough quality for assessment. Filming was conducted at a slow steady rate (approximately 5 m min^{-1}) with fixed focus, and the transect line remained in the field of view throughout. An additional light source was used so that illumination was independent of depth. The camera operator maintained a constant height of 0.5 m above the sea bed, because this distance was sufficient to observe a path of approximately 30 cm on either side of the transect line and still close enough to the seabed to be able to identify most organisms. These procedures were applied for both the diver- and ROV-collected video recordings so that results obtained by the two methods were comparable.

Environmental variables observed on the video footage were scored as an average value for all frames over 10 m intervals along boundary transects or a 5 m interval for reference transects. At the Nubeena farm there were no transect lines between F1 and F3 sample sites, and in this instance video recordings were assessed for approximately 5 m either side of the benthic infaunal sampling sites at the cage edges, and for 10 m midway between cages (which were approximately 30 m apart).

Benthic infauna were sampled in deep water at Hideaway Bay using the small Van Veen grab with a sampling area of 0.0675m^2. In the shallower sites at Nubeena, divers collected cores using 150 mm diameter PVC pipe corers to a depth of 100mm; these cores had a sampling area of 0.0177m^2. Only one method was employed at each farm

so assessments between sampling locations at each farm were consistent. Although benthic core samples were not precisely located on the video transects, the distances between core samples were much smaller than the distances over which organic wastes were dispersed, and repeated sampling over time was unlikely to be affected by previous sampling. The sediment samples with benthic infauna were sieved through a 1 mm sieve and the infauna were identified to the lowest possible taxonomic order, species where possible, and counted. Details of the benthic infaunal community structure are provided in Chapter 4.

Sample sites were classified *a priori* as unimpacted, moderately impacted and major impact, based on their proximity to stocked cages and results from previous experiments (unpublished data). All boundary and reference transects were classified as unimpacted because they were greater than 40 m away from a cage (Table 6.1). Sites between 10 and 35 m from a cage were classified as moderately impacted, and those within 0-10 m of the cage edge as experiencing a major impact.

Table 6.1. Grouping of sample sites based on the *a priori* classifications of major, moderate and unimpacted.

	Major Impact	Moderate Impact	Unimpacted
Nubeena	F1, F2 and F3 (0-10m from the cages)	midway between F1 and F2 midway between F2 and F3 F3 10-20m F35 (25-35 m from F3 cage	all boundary and reference transects
Hideaway Bay	F 0-10m	F 10-20	all boundary and reference transects

Replicate video recordings thus were obtained from all levels of impact, except moderate impact at Hideaway Bay which was only filmed satisfactorily on one occasion.

6.2.1 Video assessment

In preliminary assessments, video recordings were reviewed by three people and a large number of environmental variables were assessed for their ability to indicate change as a result of organic enrichment, and for consistency between reviewers. Several variables (substrate relief, sediment type, algal colour and burrow type) proved to be difficult to discriminate in the videos and were often ranked differently by the reviewers, consequently they were omitted from further video assessments. Initially, detailed identification of all benthic epifauna observed was undertaken but this proved to be both difficult and time consuming. Consequently, it was decided to record higher taxa (molluscs, echinoderms, crustaceans, annelids and fish), while any group/species that occurred in particularly high abundance could be noted in the comments.

Variables that were selected as potentially good indicators of change in response to farming activities were ranked according to degree of density or presence/absence (Table 6.2). Information on the quality of the video was also included to clearly distinguish between a variable being absent (zero) or unknown because of poor quality (X). However, data on debris, and on density of fish and annelids were not included in any analysis for divergent reasons. Records of debris showed impacts of marine farming other than organic enrichment. Fish were rarely observed, resulting in many zero data points that could disproportionately affect the analysis. Although other studies have shown that annelids can be important indicators of organic enrichment, annelids were never observed at the sediment surface in this study.

Table 6.2. Reference sheet for scoring video assessments.

Video Assessment Reference Sheet

Sediment colour (Only note if grey or black)	1. Black / Grey	**Debris**	1. Farm 2. Other
***Beggiatoa* cover**	1. Patchy 2. Thin Mats 3. Thick Mats	**Fishes**	1. Sparse 2. Dense
Algal cover	1. Sparse 2. Moderate 3. Dense	**Pellets / Faeces**	1. Sparse 2. Dense
		Faunal Tracks	1. Present
Burrow Density	1. Sparse 2. Moderate 3. Dense	**Gas Bubbles**	1. Present
		Crustaceans	1. Sparse 2. Dense
Echinoderms	1. Sparse 2. Dense	**Annelids**	1. Sparse 2. Dense

For the other variables, a reference collection containing representative images of each category (e.g. images of patchy, thin mats and thick mats of *Beggiatoa*) was compiled for comparison with video footage to reduce variation among reviewers. A data sheet, on which the ranking of each variable over the transect intervals could be entered, was developed to standardise data collection (Table 6.3). To maintain consistency in data collection, a single observer interpreted the videos.

Table 6.3. Data sheet for video assessment.

Video Transect Assessment Record

AREA.: **Project Code:**
Transect ID.: **Date of Assessment :**
Date of Video : **Assessed By:**
Diver / ROV Operator :

	Distance Along Transect in Metres					
	0 - 10	10 - 20	20 - 30	30 - 40	40 - 50	50 - 60
Sediment Colour						
Beggiatoa Cover						
Algal (seagrass) % Cover						
Burrow Density						
Pellet/Faeces Density						
Faunal Tracks						
Gas Bubbles						
Molluscs - Density						
Echinoderms - Density						
Crustaceans - Density						
Debris						
Fishes - Density						

Comments/ Snapshots

Video Quality: ☐ **1. Excellent**
(tick box) ☐ **2. Acceptable**
☐ **3. Unacceptable (tick one or more of the ratings box if video unacceptable)**

Rating: ☐ **1. Poor visibility/ clarity**
☐ **2. Speed of filming too fast**
☐ **3. Transect line not visible**
☐ **4. Filming too far above substrate to discern features**

Other comments:

6.2.2 Data analysis

In order to simplify comparisons of the video recordings at both farms, only the 40 –50 m section of boundary transects (around the 35 m compliance point from the lease boundary), and the 0 - 10 m section of reference transects were used in the analyses. At

Nubeena video data were collected from sample sites at the edge of farm cages (F1, F2, F3), at 10 - 20 m and 25 - 35 m on the 35 m transect extending from the F3 cage. Videos were also assessed over a 10 m interval midway between the cages (F1/2, F2/3) to provide additional information on intermediate impact. At Hideaway Bay video footage was not always successfully recorded on every occasion. At the farm F transect video footage beyond 10 m from the cage was not of acceptable quality for assessment except for F 10 - 20m at 6 months. Videos were also not successfully recorded for both the B5 and B6 transects at the 40 - 50m section at the 6 and 9 month samplings.

To investigate which variables recorded from the videos most clearly separated major, moderate and no impact site groupings, the number of sample sites where each variable occurred (regardless of ranking) in each group was recorded as a percentage of all sites in that group. The medians of the groups were compared by the non-parametric Kruskal-Wallis test (Zar, 1996).

The results of the ranked environmental variables from different sites, and benthic species abundance data were compared by multivariate analyses using the PRIMER™ software package (Carr, 1996), as described in Chapter 4. Patterns in the distribution of environmental variables observed in video recordings, and benthic infaunal assemblages, at the different sample stations were analysed using hierarchial agglomerative clustering and multidimensional scaling (MDS) ordination. Differences in species abundance, or rankings of video environmental variables, between farm cage stations and reference and boundary transect stations were tested using Analysis of Similarity (ANOSIM) techniques (Clarke and Warwick, 1994). The relationship between the benthic biotic similarity matrix and video data matrix was statistically tested using the RELATE analysis and Spearman rank correlation coefficients (Clarke and Warwick, 1994).

6.3 Results

6.3.1 Video assessment

The variables used in the multivariate analysis at Nubeena were sediment colour, *Beggiatoa* cover, algal cover, burrows, pellets and faeces, molluscs and echinoderms. Crustaceans, gas bubbles and faunal tracks were excluded because they were very rarely or never recorded. Cluster analysis separated the sites into two major groups with 47% similarity (Fig. 6.1). Group 1 consisted of heavily affected sites which were at the edge of farm cages on all sampling occasions, and one intermediate site midway between F2 and F3 at 5 months. Group 2 contained all remaining observations at intermediate as well as at unaffected sites. The position of the farm cage sites, F1, F2 and F3 at the far right of MDS plot at the five month sampling in autumn and F2 at ten months suggests that these sites were most different from the unimpacted ones. Comparison of the video results at the farm cage sites to the boundary, reference and 35 m from cage sites by ANOSIM showed a highly significant difference between the groups ($R = 0.823$, $P < 0.001$).

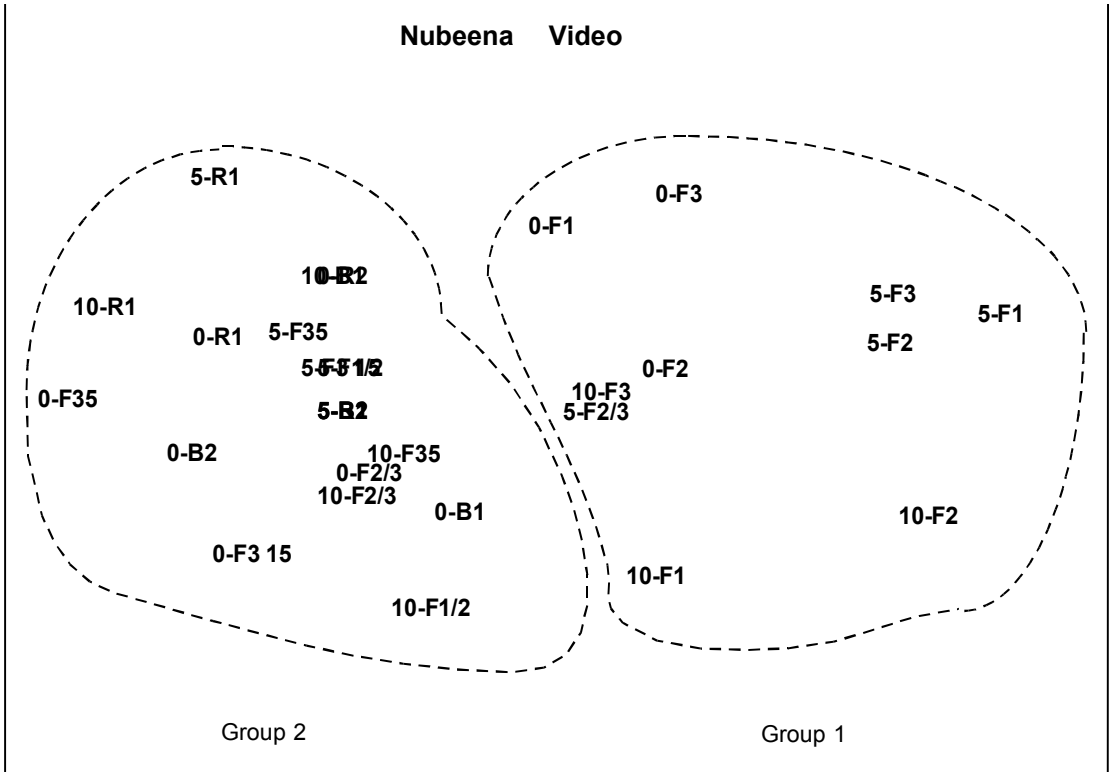


Fig. 6.1. Ordination plot (MDS) of video data at Nubeena from transects at the edge of farm cages (F1, F2, F3), between cages and 10-35m from a cage (F1/2, F2/3, F15, F35), boundaries (B1, B2) and reference sites (R1, R2). Numbers prefixed to sampling sites are sample times in months. Stress = 0.09.

At Hideaway Bay the environmental variables sediment colour, *Beggiatoa* cover, burrows, faunal tracks, pellets and faeces, molluscs, echinoderms and crustaceans were included in the data analysis. Algal cover and gas bubbles were excluded because they were not present at any of the sites.

Video records from the cage edge on four sampling occasions (3F, 6F, 9F and 11F) were grouped separately from all the other transects in the cluster analysis with similarity between the two groups of only 28%. The only other farm transect data, at 10 - 20m from the cage edge at the 6 month sampling (6F 10-20), was grouped with the boundary and reference station transects. The MDS plot (Fig. 6.2a) suggests that the farm cage sites were most impacted at 3 and 9 months and slightly less impacted at the 6 and 11 month samplings. Farm cage transects were significantly different from reference and boundary transects (ANOSIM, $R = 0.697$, $P < 0.001$).

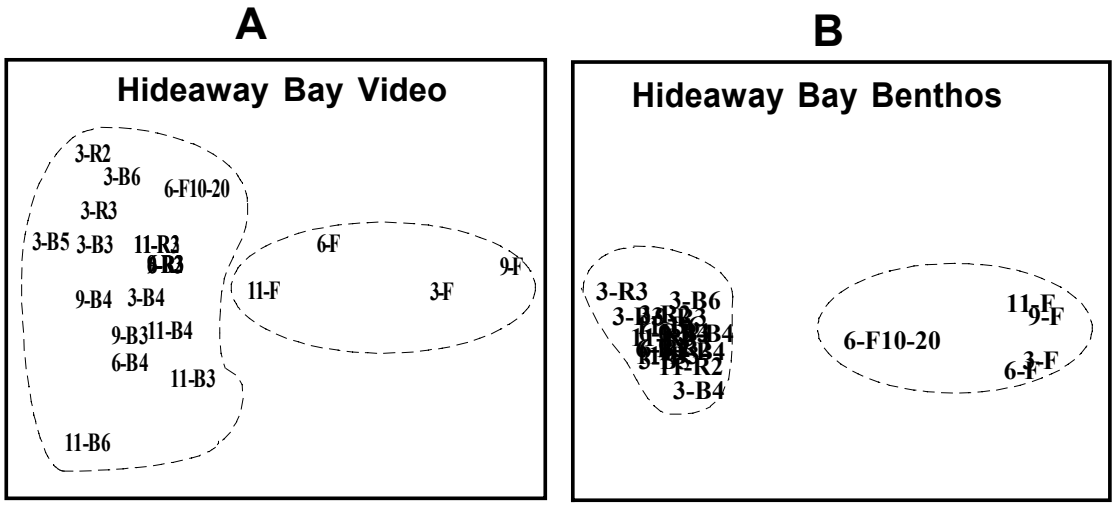


Fig. 6.2a and 6.2b. Ordination plots (MDS) based on (A) video assessments (stress = 0.11) and (B) benthic community composition (stress = 0.07), respectively, at Hideaway Bay farm cage (F), boundary (B) and reference site (R) transects. Numbers prefixed to sampling sites are sampling times in months.

6.3.2 Comparison of variables between impacted and unimpacted transects.

The variables observed on the videos which separated the impacted farm cage transects from intermediate and unimpacted transects were further investigated by tabulating the presence/absence of each variable (irrespective of the ranking) at both farm sites. Table 6.4 shows that sediment colour, *Beggiatoa* cover and pellets and faeces were present at nearly all impacted transects and absent from all unimpacted sites. Algal cover was significantly different between impacted and unimpacted sites at Nubeena, but was rarely observed at Hideaway Bay.

Table 6.4. Percentage occurrence of each environmental variable in the videos at all sites determined *a priori* as either impacted, intermediate or unimpacted. Impacted stations were 0-10m from a cage, intermediate within the range of 10-40m from a cage, and unimpacted were boundary and reference stations. Medians of each group were compared using the non-parametric Kruskal-Wallis test, *** P<0.001, ** P<0.01, * P<0.05.

	Hideaway Bay			Nubeena			
	% Impacted	% Unimpacted		% Impacted	% Intermediate	% Unimpacted	
	n=4	n=19		n=9	n=10	n=10	
Sediment Colour	100	0	***	100	0	0	***
Beggiatoa Cover	100	0	***	78	0	0	***
Algal (seagrass) Cover	0	5		33	90	100	**
Burrow Density	75	100	*	100	90	90	
Pellet/Faeces Density	100	0	***	78	10	0	***
Faunal Tracks	50	84		0	0	10	
Gas Bubbles	0	0		0	0	0	
Molluscs - density	0	26		78	100	100	
Echinoderms - density	0	37		22	90	50	*
Crustaceans - density	25	89		0	0	10	
Annelids -density	0	0		0	0	0	
Debris	0	0		22	30	0	
Fishes - density	25	21		22	50	50	

6.3.3 Comparison of video and biota assessments

MDS ordination plots of the video assessments and benthic infaunal data (at the sites where data were available from both methods) at Hideaway Bay and Nubeena (Fig. 6.2a, b and Fig. 6.3a, b, respectively) were very similar. In both cases, the gradient of impact increased from unimpacted on the left to impacted on the right. However, there were also important subtle differences. The video data separated sites at the edge of cages (F's) from all other sites, whereas the benthic data separated all sites located within 35 m of the edge of cages (F's and F35) from the boundary and reference sites.

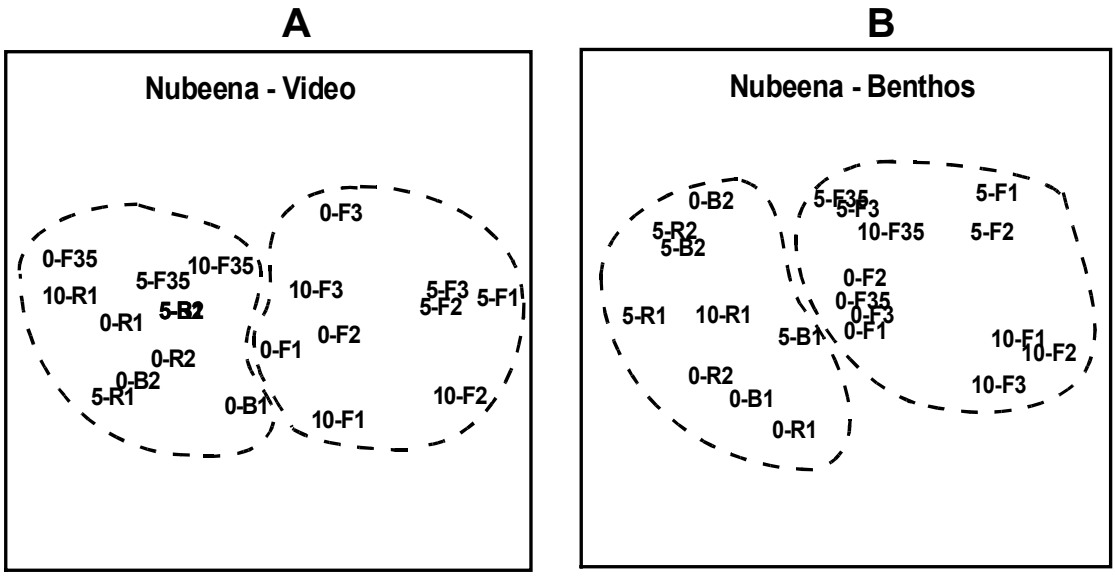


Fig. 6.3a and 6.3b. Nubeena farm ordination plots (MDS) based on (A) video assessment and (B) benthic invertebrate community composition at cage (F), boundary (B) and reference site (R) transects. Numbers prefixed to the sites identify the time of sampling (months). Stress associated with both MDS plots = 0.07.

The main grouping of sites at Hideaway Bay based on the video data (Fig. 6.2a) and on the benthic species composition (Fig. 6.2b) are very similar. However, the 'impacted group' from the benthic assessment includes the one site 10 m from the cage as well as the sites at the cage edge, whilst the 'impacted group' from the video data only includes the transects at the edge of the cage. The relationship between the biotic and video data at Hideaway Bay was, nevertheless, highly significant, (RELATE analysis, test statistic = 0.66, $P < 0.01$).

At Nubeena the MDS plot of the video assessment data for sites where benthic data were also available (Fig. 6.3a) is very similar to that shown in Fig. 6.3b. The sites at Nubeena were clearly separated into two main groups: those next to the cages and reference, boundary, and the F35 sites. The MDS plot of the macrofaunal species composition data from the same sites used in the video analysis (Fig. 6.3b) also separated the data into two groups, shown by cluster analysis to have a similarity of 20%. However, in the faunal MDS plot one group contained all the sites along the farm cage transects, including the intermediate impacted F35 site, whereas the other group consisted of all the reference and boundary stations. The biotic and video data matrices

at Nubeena were shown to be significantly related (RELATE analysis, test statistic = 0.39, P <0.01).

6.4 Discussion

The results suggest that video data can be used to separate heavily affected cage transects from unimpacted ones, but can not readily discriminate between intermediate and unimpacted transects. The main environmental variables identified in the videos which showed significant differences between impacted and unimpacted transects were sediment colour, presence of pellets and faeces, and *Beggiatoa* cover. Some variables such as algal cover were site specific; this was an important variable at Nubeena where it is normally abundant, but not at Hideaway Bay where it rarely occurs. The presence of bacterial mats has also been observed by Krost *et al.* (1994) to be an important indicator in video recordings of organic enrichment from fish cages. Angel *et al*. (1998) found that the coverage and thickness of bacterial mats, and the degree of seagrass cover were important factors recorded in diver logs for assessing benthic impacts of fish farms and these variables were given a high weighting in their fuzzy logic analysis.

Associated fauna were generally found to be poor indicators of impact, as were substrate structure and cover because densities of molluscs, fish and crustaceans were often similar at both impacted and unimpacted sites. This is surprising because the relatively immobile molluscs and crustaceans are in direct contact with the substrate, and changes in condition of the substrate would be expected to affect the associated epifauna directly. However, it proved extremely difficult to distinguish live molluscs from dead ones on the video footage, especially gastropods, and this may have affected our results. Other studies that assessed video footage on the basis of macrofaunal abundance also found video records to be of lesser value in monitoring programs than other variables. For example, GESAMP (1996) gave video a low ranking, and Cheshire *et al*. (1996) outlined the need for greater taxonomic discrimination. Future assessments should examine more closely the relationship between the epifauna and the level of organic enrichment, especially changes in species composition with increased biodeposition and whether molluscs are alive or dead.

The significant difference in densities of echinoderms between the heavily affected and intermediate transects at Nubeena warrants further investigation. At the intermediate transects the main species observed was the native starfish *Coscinasterias muricata* which was scavenging on molluscs, primarily mussels *Mytilus edulis*. These mussels are likely to have originated from the salmon cages because fouling of salmon cages by mussels is a common problem in south eastern Tasmania. Thus, fallout from farm cages may have provided an attractive food source, and attracted echinoderms. Obvious farm debris, such as ropes and rubbish, were also most prevalent at the intermediate impacted transects. Burrows, which are indicative of bioturbation, were frequently observed along all transects at both farms. Faunal tracks were also commonly recorded at transects at Hideaway Bay, but not at Nubeena because the sediment surface was hidden under algal cover. Further analyses of the data with these two parameters removed resulted in greater separation of impacted and unimpacted sites and we suggest the categories of burrows and faunal tracks need to be more carefully defined in future assessments.

The overall grouping of sites achieved by video and benthic assessments was similar, although the intermediate impacted sites were grouped with the impacted sites according to the benthos samples, and with the unimpacted sites according to video. The benthic infaunal assessments indicated that the species composition at these intermediate sites showed more commonality with that at the organically enriched cage stations than with the reference and boundary stations. By contrast, Rumohr and Karakassis (1999) found that abundance of benthic infauna was not significantly correlated with the physical and biotic characteristics observed in photographs collected using sediment profiling imagery (SPI) techniques. Instead, they suggested that the two methods were complementary, with the benthic fauna being sensitive to anoxic events, and the SPI data to physical disturbances of the seabed, such as fishing activity.

The MDS plot of the video data for Nubeena indicates that the most degraded conditions generally occurred at the 5 month sampling whilst the benthic assessment suggests that they occurred at 10 months. Thus, the video data suggest that an improvement in environmental conditions had occurred after the cages had been fallowed for seven weeks. The benthic assessment implied, however, a continuation of degraded conditions. This difference may be related to the fact that the video data grouped the sample sites on the basis of visual characteristics at the sediment surface which are clearly indicative of organic enrichment, whilst the benthic community structure reflected conditions within the sediment. Recovery after an organic enrichment event has been shown to occur more rapidly at the surface than within the sediments (Pearson and Rosenberg, 1978), and this may account for the differences in site groupings between the video and the benthic assessment. The video variable shown to be most closely associated with the benthic community structure (data not included) was sediment colour, a variable more indicative of the conditions within the sediment. Differences in the timing of sediment condition were also apparent between the video and biotic data at Hideaway Bay. However, the relationship between sediment health and cage stocking was not as clear because fish were routinely moved on and off this site at 1-3 month intervals.

Our results thus suggest that video assessment is most useful as an indicator of sediment condition when evaluated regularly and in conjunction with an ongoing source of organic enrichment. Under these conditions video assessment most closely relates to benthic community status. However, video assessment may not be as useful in assessing sediment condition when the source of organic enrichment has been removed or reduced, and the sediments are in recovery phase. The results also emphasise the need for caution in assessing sediment condition using video footage because although the sediment may appear healthy at the surface, it could be degraded underneath. Further research is required to refine video assessments of recovery sites and of intermediate levels of organic enrichment.

Although quantitative assessment of video recordings can be used as an objective measure of environmental change around fish farms, video assessments only detect major changes. Therefore, other environmental variables such as benthic infauna composition and physical/chemical measures should also be included in any routine environmental monitoring program. We also acknowledge that these analyses have been conducted on limited data and they will need to be reassessed and refined as more video data are analysed, particularly from sites with different environmental features.

7. Summary and Recommendations for a Monitoring Program

This research on assessing techniques for environmental monitoring of localised impacts of salmonid farms has addressed several issues:

(i) Which environmental variables/techniques are good indicators of organic enrichment from salmon farms and would be suitable for a monitoring program, i.e. are practicable, inexpensive and scientifically credible.

(ii) What levels of impact can be detected.

(iii) How many benthic infaunal samples are required to reliably assess environmental impact.

Three other issues were also partially addressed:

(iv) How many reference (control) sites are required.

(v) Where should samples be collected within and outside the lease area.

(vi) When during the year and how often should samples be collected.

7.1 Research Results In Relation To Developing A Monitoring Program

7.1.1 Physical-chemical variables

- Redox proved to be a simple, quick and inexpensive indicator of major organic enrichment provided it was carefully measured using standard procedures. Redox values below zero indicated major impact and suggested that additional evaluation of the severity of the impact was required.

- Sediment particle size was found to be an important variable to measure in environmental monitoring because it was indicative of current speeds and could influence other environmental variables such as infaunal community structure.

- Total organic matter, as measured by Loss on Ignition, was highly correlated with sediment particle size, and the research results implied that it was a poor indicator of organic enrichment.

- Stable isotopes of N and % elemental N were indicators of major impact only. $\delta^{15}N$ was found to be useful as a tracer of salmon wastes only when the organic loading was high. Thus the cost-effectiveness of measuring stable isotopes of N would need to be evaluated against other measures of organic enrichment.

- Stable isotopes of C and % organic C were highly variable and inconsistent, and are not recommended for a monitoring program until standardised analytical methods are developed.

7.1.2 Benthic infaunal community structure

- Benthic infauna abundance and community composition were found to be sensitive measures of organic enrichment because they could be used to characterise sites as having had major, moderate or no impact. These variables also showed a gradation of effect with the duration and intensity of farming.

- The results show that analysis of the benthic infaunal data in several ways provides a better overall picture of the level of impact. Both univariate and multivariate analyses of benthic infaunal data were found to be suitable. K-dominance curves, however, were considered to be less useful because many graphs were required to present the data, and the curves did not distinguish between different sources of impact.

- Taxonomic discrimination to family level was found to be sufficient to identify major organic enrichment. However, identification to species level provided more subtle information on the state of the sediment ecosystem.

Changes in benthic biota which indicated differing levels of impact included:

- *Total Abundance (Numbers per m^2)*

  An increase of x10 implied moderate impact and a more comprehensive assessment may be required. An increase of x20, or a defaunated community, suggested that a major environmental impact had occurred.

- *Species composition*

  Dominance of the species composition of a site by *Capitella sp* indicated that a moderate impact had occurred, and *Capitella* sp comprising more than 50% of the total number of individuals suggested major environmental impact.

- *Shannon Diversity Index - Inverse Simpson Index*

  A Shannon index of < 2 indicated that a moderate impact had occurred, and < 1 major impact. Inverse Simpson Index of < 6 implied moderate environmental impact and < 1 major impact.

- *MDS plots*

  Any site that lay within the 90% confidence kernel of an impacted cluster grouping was also impacted.

- *Species Richness (Number of species per sample)*

  A reduction in the number of species by 50% generally indicated that an impact had occurred. Very low species number implied severe impact.

7.1.3 Number of faunal samples required

The number of benthic infaunal samples required to reliably assess an impact at 35 m from each of 4 farm boundaries was determined by power analysis to be:

- 4 reference sites, 4 boundary sites, 3 sampling points at each boundary and reference site, and 1 sample at each sampling point, (i.e. single samples to be collected approximately 20 m apart at 3 points on each of 4 boundaries and at each of the 4 reference sites, a total of 24 samples.)

7.1.4 Video assessment

- Video recording of the benthic environment was found to be a very valuable technique for environmental monitoring because it provided an on the spot and readily interpretable permanent record. However, it was not as sensitive as benthic infauna as a measure of organic enrichment, and generally only discriminated major impact.

- Analysis of the video data using the quantitative techniques developed and multivariate analysis simplified the assessment and reduced the level of subjectivity in the interpretations.

- Important indicators of major impact observed in the video recordings were dense *Beggiatoa* mats, black sediment and dense pellets and faeces. Algal cover was an important indicator at sites where it normally occurred.

7.2 Recommendations For A Monitoring Program

7.2.1 Environmental variables

The results indicate that several environmental variables should be measured in a farm monitoring program because no one variable was consistently representative of all environmental impacts. Benthic infauna, video assessments and redox were found to be the most reliable and useful indicators of organic enrichment at the two farms investigated.

7.2.2 Monitoring sites

A monitoring program to meet legislative requirements of no unacceptable impact occurring 35 m from the lease area should be developed separately from a monitoring program to assist farm management protocols and to develop appropriate fallowing

regimes. To combine the two together runs the risk of neither monitoring program achieving the required level of sampling to provide reliable outcomes.

Suggested monitoring sites to ensure compliance with the legislation at a standard 20 ha farm are:

- Two sites at fixed locations 35 m from the lease boundary, one upstream and one downstream of the farm. With no between site variation, these sites will have greater power to detect change over time.

- Two sites 35 m from the upstream and downstream boundary that are chosen because of the likelihood of a relatively high level of impact.

- If the direction of current flow and hence spread of impact is uncertain, an additional three sites at 35 m outside the other two farm boundaries.

- At least three reference sites located approximately 500m from the farm with environmental conditions, especially sediment particle size, similar to the farm.

The data collected at these sites should be analysed for changes over time, and for differences between reference and compliance sites.

7.2.3 Timing of monitoring

Several environmental variables have indicated seasonal changes, which suggests that monitoring at each farm should be conducted at the same time of year. Redox values were lowest in autumn, so this may be the most appropriate time of year to conduct monitoring. However, additional seasonal comparisons will be required to verify this.

Because video and redox are quick and inexpensive measures of environmental impact, it is suggested that they are routinely monitored, preferably twice a year. If these measures indicate an impact, then more comprehensive monitoring should be conducted to assess the intensity of the impact.

These variables, however, generally only show major effects of organic enrichment, and more subtle environmental change is detected from analysis of the benthic infaunal community structure. To assess the health of the sediment ecosystem, it is recommended that benthic infauna is also monitored, preferably at least once every two years.

7.2.4 Monitoring techniques

It is important that all monitoring is conducted using the same techniques and equipment according to detailed specifications so that spatial and temporal comparisons of monitoring results are valid. This is particularly important for benthic assessments where minor changes, such as mesh bag material, can produce major changes in the results.

7.2.5 Long Term Monitoring Program

These recommendations are based on results obtained at two farms. The monitoring program should be regularly assessed and revised as more data become available.

In the longer term, monitoring programs should be tailored to the environmental conditions and intensity of farming at each site. As more data become available from each farm, it should be possible to develop site-specific monitoring programs that are based on the production of fish from the farm, and the environmental sensitivity of the site.

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References

Angel, D. L., Krost, P. and Silvert, W. L. (1998). Describing benthic impacts of fish farming with fuzzy sets: theoretical background and analytical methods. *Journal of Applied Ichthyology,* Vol. **14**, 1-8.

Barnard, J. K. and Drummond, M. M. (1978). Gammaridean Amphipoda of Australia, Part III: The Phoxocephalidae. Smithsonian Contributions to Zoology No.245. Smithsonian Institution Press, Washington.

Bayne, B. L., Addison, R. F., Capuzzo, J. M., Clarke, K. R., Gray, J. S., More, M. N. and Warwick, R.M. (1988). An overview of the GEEP workshop. *Marine Ecology Progress Series*, Vol.**46**, 235-243.

Beukema, J. J. (1988). An evaluation of the ABC-method (abundance/biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden Sea. *Marine Biology,* Vol. **99**, 425-433.

Black, K. D., Kiemer, M. C. B. and Ezzi, I. A. (1996). Aquaculture and Sea Lochs. In *Aquaculture and Sea Lochs.* (ed. K. D. Black), pp. 16-26. Oban, Scotland: The Scottish Association for Marine Science.

Bowman, M. F. and Bailey, R. C. (1997). Does taxonomic resolution affect the multivariate description of the structure of freshwater benthic macroinvertebrate communities? *Canadian Journal of Fisheries and Aquatic Sciences,* Vol. **54** (8), 1802-1807.

British Columbia Environmental Assessment Office (1998). Final Salmon Aquaculture Report [Online]. Available: http://www.eao.gov.bc.ca/PROJECT/AQUACULT/ SALMON/report/toc.htm [1999, June 24].

Brower, J. E., Zar, J. H. and von Ende, C. N. (1990). Field and laboratory methods for general ecology. W.C. Brown, Dubuque, USA. 237p.

Brown, J. R., Gowen, R. J. and McLusky, D. S. (1987). The effect of salmon farming on the benthos of a Scottish sea loch. *Journal of Experimental Marine Biology and Ecology*, Vol. **109**, 39-51.

Byers, S. C., Mills, E. L. and Stewart, P. L. (1978). A comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. *Hydrobiologia,* Vol. **58**, 43-47.

Carr, M. R. (1996). PRIMER User Manual. (Plymouth Routines in Multivariate Ecological Research). Plymouth Marine Laboratory, United Kingdom.

Chang, B. D. and Thonney, J. P. (1993). Overview and environmental status of the New Brunswick salmon culture industry. *Bulletin of Aquaculture Association of Canada*, Vol. **92-93**, 61-63.

Cheshire, A., Westphalen, G., Smart, A., and Clarke, S. (1996). Investigating the environmental effects of sea-cage tuna farming. II. The effects of sea-cages. Report to FRDC on Project 94/091. Department of Botany, University of Adelaide, South Australia.

Clarke, K. R. and Warwick, R.M. (1994). Changes in marine communities: an approach to statistical analysis and interpretation. Natural Environment Research Council, UK, 144pp.

Cochrane, S. (1995). Assessment/development of methods for monitoring of sediments under fish farms. Tromso, Norway: Akvaplan-niva.

Cochrane, S. J. and Pearson, T. H. (1995). Evaluation of methods and development of procedures for environmental monitoring of fish farms. Main Report from Akvaplan-Niva, Tromso, Norway.

Codling, I. D., Doughty, R., Hunter, J., Henderson, A. and Naismith, I. (1995). Strategies for monitoring sediments and fauna around cage fish farms. Scotland and Northern Ireland Forum for Environmental Research. Report No. SR 4018.

Craft, C. B., Seneca, E. D. and Broome, S. W. (1991). Loss on ignition and Kjeldahl digestion for estimating organic carbon and total nitrogen in estuarine marsh soils: Calibration with dry combustion. *Estuaries,* Vol. **14**, 175-179.

Crawford, C. M., Mitchell, I. M. and Macleod, C. K. A. (2001). Video assessment of environmental impacts of salmon farms. *ICES Journal of Marine Science,* Vol. **58**, 445-452.

CSIRO Huon Estuary Study Team (2000). Huon Estuary Study - environmental research for integrated catchment management and aquaculture. Final report to Fisheries Research and Development Corporation. Project number 96/28, June 2000. CSIRO Division of Marine Research, Marine Laboratories, Hobart.

Day, R. W. and Quinn, G. P. (1989). Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs,* Vol. **59**, 433-463.

DPIF. (1996a). Marine Farming Development Plans for Tasmania, Huon River and Port Esperance. Department of Primary Industry and Fisheries (DPIF). Hobart, Tasmania.

DPIF. (1996b). Marine Farming Development Plans for Tasmania, Tasman Peninsular and Norfolk Bay. Department of Primary Industry and Fisheries (DPIF). Hobart, Tasmania.

Drake, P. and Arios, A. M. (1997). The effect of aquaculture practices on the benthic macroinvertebrate community of a lagoon system in the Bay of Cadiz (southwestern Spain). *Estuaries*, Vol. **20** (4), 677-688.

Edgar, G. J., Barrett, N. S. and Last, P. R. (1999). The distribution of macroinvertebrates and fishes in Tasmanian estuaries. *Journal of Biogeography,* Vol. **26** (6), pp. 1169-1189.

Ellis, D. (1985). Taxonomic sufficiency in pollution assessment. *Marine Pollution Bulletin*, Vol. **16** (12), 459.

Fairweather, P. G. (1991). Statistical power and design requirements for environmental monitoring. *Australian Journal of Marine and Freshwater Research*, Vol. **42**, 555-67.

Ferraro, S. P. and Cole, F. A. (1990). Taxonomic level sufficient for assessing a moderate impact on the Southern Californian Bight macrobenthos. *Marine Ecology Progress Series*, Vol. **67**, 251-262.

Ferraro, S. P. and Cole, F. A. (1992). Taxonomic level sufficient for assessing a moderate impact on macrobenthic communities in Puget Sound, Washington, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. **49**, 1184-1188.

GESAMP. (1996). (IMO/FAO/Unesco/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution). Monitoring the ecological effects of coastal aquaculture wastes. *GESAMP Reports and Studies,* **57,** 38p.

Gowen, R. J., Brown, J. R., Bradbury, N. B. and McLusky, D. S. (1988). Investigation into benthic enrichment, hypernutrification and eutrophication associated with mariculture in Scottish coastal waters (1984-1988). Dept. Biological Science, University of Stirling. p.289.

Gowen, R. J. (1991). Aquaculture and the environment. In *European Aquaculture Society Special Publication No. 16*, Vol. 16 (ed. N. De Pauw and J. Joyce). Gent, Belgium: European Aquaculture Society.

Gray, J. S., Clarke, K. R., Warwick, R. M. and Hobbs, G. (1990). Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields, North Sea. *Marine Ecology Progress Series*, Vol. 66, 285-299.

Green, R. H. (1989). Power analysis and practical strategies for environmental monitoring. *Environmental Research*, Vol. **50**, 195-205.

Greiser, N. and Faubel, A. (1988). Biotic Factors. In *Introduction to the Study of Meiofauna.* (ed. Higgins, R. P. and Theil, H.). Smithsonian Institution Press, Washington, pp. 79-114.

Hansen, P. K., Pittman, K. and Ervik, A. (1990). Effects of organic waste from marine fish farms on the seabottom beneath the cages. *International Council for the Exploration of the Sea,* **C.M. 1990/F: 34**, 1-15.

Hargrave, B. T., Duplisea, D. E., Pfeiffer, E. and Wildish, D. J. (1993). Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. *Marine Ecology Progress Series*, Vol. **96**, 249-257.

Hargrave, B. T., Phillips, G. A., Doucette, L. I., White, M. J., Milligan, T. G., Wildish, D. J. and Cranston, R. E. (1997). Assessing benthic impacts of organic enrichment from marine aquaculture. *Water, Air, and Soil Pollution,* Vol. **99**, (1-4) 641-650.

Heinig, C. S. (1996). The Maine Department of Marine Resource's Finfish Aquaculture Monitoring Program (FAMP) 1992-1995. A report to the Joint Standing Committee on Marine Resources Second Session of the 117th Maine State Legislature. Maine Department of Marine Resources, Augusta, Maine.

Henderson, A. R. and Ross, D. J. (1995). Use of macrobenthic infaunal communities in the monitoring and control of the impact of marine cage fish farming. *Aquaculture Research*, Vol. **26**, 659-678.

Holmer, M. and Kristensen, E. (1992). Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. *Marine Ecology Progress Series*, Vol. **80**, 191-201.

Horwitz, P. and Blake, G. (1992). The benthic macrofauna of sludge-affected sediments in the Derwent estuary, Southern Tasmania. *Papers and Proceedings of the Royal Society of Tasmania*, Vol. **126**, 67-72.

James, R. J., Lincoln Smith, M. P. and Fairweather, P. G. (1995). Sieve mesh-size and taxonomic resolution needed to describe natural spatial variation of marine macrofauna. *Marine Ecology Progress Series*, Vol. **118**, 187-189.

Johannessen, P. J., Botnen, H. B. and Tvedten, Ø. (1994). Macrobenthos: Before, during and after a Fish Farm. *Aquaculture and Fisheries Management*, Vol. **25**, 55-66.

Karakassis, I., Tsapakis, M. and Hatziyanni, E. (1998). Seasonal variability in sediment profiles beneath fish farm cages in the Mediterranean. *Marine Ecology Progress Series*, Vol. **162**, 243-252.

Karakassis, I., Hatziyanni, E., Tsapakis, M., Plaiti, W. (1999). Benthic recovery following cessation of fish farming: a series of successes and catastrophes. *Marine Ecology Progress Series*, Vol. **184**, 205-218.

Karakassis, I., Tsapakis, M., Hatziyanni, E., Papadopoulou, K. N. and Plaita, W. (2000). Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES Journal of Marine Science*, Vol. **57**, 1462-1471.

Kristensen, E. and Anderson, F. O. (1987). Determination of organic carbon in marine sediments: a comparison of two CHN-analyzer methods. *Journal of Experimental Marine Biology and Ecology,* Vol. **109**, 15-23.

Krost, P., Chrzan, T., Schomann, H. and Rosenthal, H. (1994). Effects of a floating fish farm in Kiel Fjord on the sediment. *Journal of Applied Ichthyology,* Vol. **10**, 353-361.

Kupka-Hansen, P., Lunestad, B. T. and Samuelsen, O. B. (1991). Environmental effects of antibiotic/chemotherapeutics from aquaculture. pp.178-179. In DePauw N. and Joyce, J. (eds). Aquaculture and the environment. Short communications and abstracts. European Aquaculture Special Publication 14. p.332.

Lambshead, P. J. D., Platt, H. M. and Shaw, K. M. (1983). The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *Journal of Natural History*, Vol. **17**, 859-874.

Lim, S. (1991). Environmental impact of salmon farming on the benthic community in the Bay of Fundy. *Bulletin of the Aquaculture Association of Canada*, Vol. **91**-3, 126-128.

Lu, L. and Wu, R. S. S. (1998). Recolonization and succession of marine macrobenthos in organic-enriched sediment deposited from fish farms. *Environmental Pollution*, Vol. **101**, 241-251.

Macleod, C. M. (2000). Techniques for farm-based assessment of sediment health associated with the commercial culture of Atlantic salmon (*Salmo salar* L.) in Tasmania. MSc Thesis, University of Tasmania, Australia.

McGhie, T. K., Crawford, C. M., Mitchell, I. M. and O'Brien, D. (2000). The degradation of fish-cage waste in sediments during fallowing. *Aquaculture,* Vol. **187**, 351-366.

Midwood, A. J. and Boutton, T. W. (1998). Soil carbonate decomposition by acid has little effect on $d^{13}C$ of organic matter. *Soil Biology and Biochemistry,* Vol. **30**, 1301-1307.

Mitchell, I. M., Crawford, C. M. and Macleod, C. K. (1997). Environmental Assessment of Marine farm #93 Huon Aquaculture Company P/L, Hideaway Bay. Marine Research Laboratories, DPIWE. Taroona, Tasmania.

Nieuwenhuize, J., Maas, Y. E. M. and Middelburg, J. L. (1994). Rapid analysis of organic carbon and nitrogen in particulate materials. *Marine Chemistry,* Vol. **45**, 217-224.

O'Connor, B. D. S., Costelloe, J., Keegan, B. F. and Rhoads, D. C. (1989). The use of REMOTS technology in monitoring coastal enrichment resulting from mariculture. *Marine Pollution Bulletin*, Vol. **20** (8), 384-390.

Parsons, T. R., Takahashi, M. and Hargrave, B. (1977). Biological oceanographic processes. (Second Edition). Oxford: Pergamon Press.

Pearson, T. H. and Rosenberg, R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology: an Annual Review*, Vol. **16**, 229-311.

Pearson, T. H. and Stanley, S. O. (1979). Comparative measurements of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. *Marine Biology*, Vol. **53**, 371-379.

Pohle, G. and Frost, B. (1997). Establishment of standard benthic monitoring sites to assess long-term ecological modification and provide predictive sequence of benthic community succession in the inner Bay of Fundy, New Brunswick. Final report. New Brunswick Department of Fisheries and Aquaculture, St. Andrews, N.B.

Rosenberg, R. (1976). Benthic faunal dynamics during succession following pollution abatement in a Swedish estuary. *OIKOS*, Vol. **27**, 414-427.

Rosenthal, H. and Rangeley, R. W. (1988). The effect of salmon cage culture on the benthic community in a largely enclosed bay (Dark Harbour, Grand Manan Island, NB, Canada), pp.207-223. In Rosenthal, H. and Lillelund, K. (eds) Fish health protection strategies. Bundesministerium für Forschung und Technologie, Hamburg, Germany.

Rumohr, H., and Karakassis, I. (1999). Comparison of multivariate patterns: different taxonomic levels in macrofaunal analysis versus sediment profiling imagery (SPI). *Marine Ecology Progress Series,* Vol. **190**, 125-132.

SEPA (n.d.). Fish Farming Manual [on line]. Available: http://www.sepa.org.uk/publications/fishfarmmanual/index.htm [1999, July 11].

Schmitt, R. J. and Osenberg, C. W. (1996). *Detecting Ecological Impacts*. Academic Press, Inc., California.

Simpson, E. H. (1949). Measurement of diversity. *Nature*, Vol. **163**, 688.

Somerfield, P. J. and Clarke, K. R. (1995). Taxonomic levels, in marine community studies, revisited. *Marine Ecology Progress Series*, Vol. **127**, 113-119.

Thornton, S. F. and McManus, J. (1994). Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary, Scotland. *Estuarine and Coastal Shelf Science,* Vol. **38**, 219-233.

Tsutsumi. (1995). Impact of fish net pen culture on the benthic environment of a cove in South Japan. *Estuaries,* Vol. **18**, 108-115.

Warwick, R. M. (1988a). Effects on community structure of a pollutant gradient – summary. *Marine Ecology Progress Series*, Vol. **46**, 207-211.

Warwick, R. M. (1988b). The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Marine Pollution Bulletin*, Vol. **19**, 259-268.

Warwick, R. M. (1988c). Analysis of community attributes of the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. *Marine Ecology Progress Series*, Vol. **46**, 167-170.

Warwick, R. M., Platt, H. M., Clarke, K. R., Agard, J. and Gobin, J. (1990). Analysis of macrobenthic and meiobenthic community structure in relation to pollution and disturbance in Hamilton Harbour, Bermuda. *Journal of Experimental Marine Biology and Ecology*, Vol. **138**, 119-142.

Warwick, R. M. (1993). Environmental impact studies on marine communities: pragmatical considerations. *Australian Journal of Ecology*, Vol. **18**, 63-80.

Weston, D. P. (1990). Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Marine Ecology Progress Series*, Vol. **61**, 233-244.

Wildish, D. J., Akagi, H. M., Hamilton, N. and Hargrave, B. T. (1999). A recommended method for monitoring sediments to detect organic enrichment from mariculture in the Bay of Fundy. *Canadian Technical Report of Fisheries and Aquatic Sciences,* Vol. **2286**, 1-31.

Wildish, D. J., Hargrave, B. T. and Pohle,G. T. (2001). Cost-effective monitoring of organic enrichment resulting from salmon mariculture. *ICES Journal of Marine Science*, Vol. **58**, 363-368.

Woodward, I. O., Gallagher, J. B., Rushton, M. J., Machin, P. J. and Mihalenko, S. (1992). Salmon farming and the environment of the Huon estuary, Tasmania. Technical Report No.45. Department of Primary Industry, Fisheries and Energy, Tasmania, Australia. p.58.

Wu, R. S. S., Lam, K. S., MacKay, D. W., Lau, T. C. and Yam, V. (1994). Impact of marine fish-farming on water quality and bottom sediment: a case study in the sub-tropical environment. *Marine Environmental Research*, Vol. **38**, 115-145.

Wu, R. S. S. (1995). The environmental impact of marine fish culture: towards a sustainable future. *Marine Pollution Bulletin,* Vol. **31,** 159-166

Ye, L., Ritz, D. A., Fenton, G. E. and Lewis, M. E. (1990). Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis. *Journal of Experimental Marine Biology and Ecology,* Vol. **145**, 161-174.

Zar, J. H. (1996). Biostatistical Analysis, 3rd edition. New Jersey, USA: Prentice-Hall International Inc.

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APPENDIX 1: POWER TO DETECT INCREASED POLLUTION AT A SINGLE FARM SITE, AVERAGED ACROSS FARM SITES, AT A FARM SITE COMPARED WITH CONTROL SITES, AND AT FARM SITES COMPARED WITH CONTROL SITES FOR THE FOLLOWING:

Appendix 1a: Total abundance of benthic infauna data at *four* control sites and *four* farm sites.

Appendix 1b: Total species of benthic infauna data at four control sites and four farm sites.

Appendix 1a.

Analysis for total abundance data (log)

Variance components	
Between sites	0.0087
Between points	0.02084
Between samples	0.03967
Type 1 error rate	0.05
No. of control sites	4
Critical *t*	3.18
No. of farm sites	4

sd of site reading

Number of samples per point	Number of points per site 1	2	3	4
1	0.26	0.20	0.17	0.15
2	0.22	0.17	0.15	0.14
3	0.21	0.16	0.14	0.13
4	0.20	0.16	0.14	0.13

Power to detect increased pollution at a single farm site
(Testing for evidence of an increase at a site over time
using within control site variance as the yardstick of non-polluting variation)

Number of points per site	Number of samples per point	sd	Size of difference / cut-off point	0.301 (log 2)	0.477 (log 3)	0.6021 (log 4)	0.699 (log 5)
1	1	0.2630779	0.837	0.07	0.13	0.22	0.32
1	2	0.2222049	0.707	0.08	0.19	0.33	0.49
1	3	0.206793	0.658	0.09	0.22	0.40	0.57
1	4	0.1986391	0.632	0.10	0.25	0.44	0.62
2	1	0.1973702	0.628	0.10	0.25	0.45	0.63
2	2	0.1704039	0.542	0.13	0.36	0.63	0.79
2	3	0.1604109	0.51	0.14	0.42	0.70	0.84
2	4	0.1551733	0.494	0.15	0.46	0.73	0.86
3	1	0.1699117	0.541	0.13	0.37	0.63	0.79
3	2	0.1491923	0.475	0.16	0.51	0.77	0.89
3	3	0.1416137	0.451	0.18	0.57	0.82	0.91
3	4	0.1376681	0.438	0.20	0.60	0.84	0.92
4	1	0.1543616	0.491	0.15	0.47	0.74	0.86
4	2	0.1373636	0.437	0.20	0.60	0.84	0.92
4	3	0.1312091	0.418	0.22	0.66	0.87	0.94
4	4	0.128021	0.407	0.23	0.69	0.89	0.95

Appendix 1a cont.

Power to detect increased pollution averaged across farm sites
(Testing for evidence of an average increase over time
using within control site variance as the yardstick of non-polluting variation)

			Size of difference	0.301	0.477	0.6021	0.699
Number of points per site	Number of samples per point	se mean	cut-off point	(log 2)	(log 3)	(log 4)	(log 5)
1	1	0.131539	0.419	0.22	0.66	0.87	0.94
1	2	0.1111024	0.354	0.33	0.83	0.94	0.97
1	3	0.1033965	0.329	0.40	0.88	0.96	0.98
1	4	0.0993196	0.316	0.44	0.90	0.97	0.98
2	1	0.0986851	0.314	0.45	0.90	0.97	0.99
2	2	0.085202	0.271	0.63	0.95	0.98	0.99
2	3	0.0802055	0.255	0.70	0.97	0.99	0.99
2	4	0.0775866	0.247	0.73	0.97	0.99	0.99
3	1	0.0849559	0.27	0.63	0.95	0.99	0.99
3	2	0.0745961	0.237	0.77	0.98	0.99	1.00
3	3	0.0708069	0.225	0.82	0.98	0.99	1.00
3	4	0.068834	0.219	0.84	0.98	0.99	1.00
4	1	0.0771808	0.246	0.74	0.97	0.99	1.00
4	2	0.0686818	0.219	0.84	0.98	0.99	1.00
4	3	0.0656046	0.209	0.87	0.99	1.00	1.00
4	4	0.0640105	0.204	0.89	0.99	1.00	1.00

Appendix 1a cont.

Power to detect increased pollution at a farm site compared with control sites
(Comparing increase at one farm site with mean increase of control sites
using within control site variance as the yardstick of non-polluting variation)

			Size of difference	0.301	0.477	0.6021	0.699
Number of points per site	Number of samples per point	se mean	cut-off point	(log 2)	(log 3)	(log 4)	(log 5)
1	1	0.2941301	0.936	0.06	0.11	0.17	0.24
1	2	0.2484326	0.791	0.07	0.15	0.25	0.37
1	3	0.2312016	0.736	0.08	0.17	0.30	0.44
1	4	0.2220853	0.707	0.08	0.19	0.33	0.49
2	1	0.2206666	0.702	0.08	0.19	0.34	0.49
2	2	0.1905174	0.606	0.10	0.27	0.49	0.67
2	3	0.1793449	0.571	0.11	0.32	0.56	0.74
2	4	0.173489	0.552	0.12	0.35	0.60	0.77
3	1	0.1899671	0.605	0.10	0.27	0.50	0.67
3	2	0.166802	0.531	0.13	0.38	0.65	0.81
3	3	0.1583289	0.504	0.15	0.44	0.71	0.85
3	4	0.1539176	0.49	0.15	0.47	0.74	0.87
4	1	0.1725815	0.549	0.12	0.35	0.61	0.78
4	2	0.1535771	0.489	0.15	0.47	0.74	0.87
4	3	0.1466963	0.467	0.17	0.53	0.79	0.89
4	4	0.1431318	0.456	0.18	0.55	0.81	0.91

Appendix 1a cont.

Power to detect increased pollution at farm sites compared with control sites
(Comparing mean of farm sites with mean of control sites
using within control site variance as the yardstick of non-polluting variation)

			Size of difference	0.301	0.477	0.6021	0.699
Number of points per site	Number of samples per point	se mean	cut-off point	(log 2)	(log 3)	(log 4)	(log 5)
1	1	0.1860242	0.592	0.06	0.22	0.53	0.76
1	2	0.1571226	0.5	0.09	0.42	0.79	0.91
1	3	0.1462247	0.465	0.11	0.54	0.86	0.95
1	4	0.1404591	0.447	0.12	0.61	0.89	0.96
2	1	0.1395618	0.444	0.12	0.62	0.90	0.96
2	2	0.1204938	0.383	0.20	0.82	0.96	0.98
2	3	0.1134277	0.361	0.25	0.88	0.97	0.99
2	4	0.1097241	0.349	0.29	0.90	0.98	0.99
3	1	0.1201457	0.382	0.20	0.83	0.96	0.98
3	2	0.1054949	0.336	0.34	0.92	0.98	0.99
3	3	0.100136	0.319	0.41	0.94	0.99	0.99
3	4	0.097346	0.31	0.45	0.95	0.99	0.99
4	1	0.1091501	0.347	0.30	0.90	0.98	0.99
4	2	0.0971307	0.309	0.46	0.95	0.99	0.99
4	3	0.0927789	0.295	0.53	0.97	0.99	1.00
4	4	0.0905245	0.288	0.57	0.97	0.99	1.00

Appendix 1b.

Analysis for total species data (log)

Variance components	
Between sites	0.00357
Between points	0.0173
Between samples	0.02381
Type 1 error rate	0.05
No. of control sites	4
Critical *t*	3.18
No. of farm sites	4

sd of site reading

Number of samples per point	Number of points per site 1	2	3	4
1	0.21	0.16	0.13	0.12
2	0.18	0.13	0.12	0.10
3	0.17	0.13	0.11	0.10
4	0.16	0.12	0.11	0.10

Power to detect increased pollution at a single farm site
(Testing for evidence of an increase at a site over time
using within control site variance as the yardstick of non-polluting variation)

Number of points per site	Number of samples per point	sd	Size of cut-off point	0.301 (log 2)	0.477 (log 3)	0.6021 (log 4)	0.699 (log 5)
1	1	0.2113764	0.673	0.09	0.21	0.38	0.55
1	2	0.1810387	0.576	0.11	0.31	0.55	0.73
1	3	0.1697253	0.54	0.13	0.37	0.63	0.79
1	4	0.1637758	0.521	0.14	0.40	0.67	0.82
2	1	0.1553222	0.494	0.15	0.46	0.73	0.86
2	2	0.1348054	0.429	0.21	0.63	0.86	0.93
2	3	0.1272334	0.405	0.24	0.69	0.89	0.95
2	4	0.1232731	0.392	0.26	0.73	0.91	0.96
3	1	0.1314281	0.418	0.22	0.66	0.87	0.94
3	2	0.1153473	0.367	0.30	0.79	0.93	0.97
3	3	0.1094633	0.348	0.35	0.84	0.95	0.98
3	4	0.1063994	0.339	0.37	0.86	0.96	0.98
4	1	0.1176754	0.374	0.29	0.78	0.93	0.96
4	2	0.1042653	0.332	0.39	0.87	0.96	0.98
4	3	0.099394	0.316	0.44	0.90	0.97	0.98
4	4	0.0968665	0.308	0.47	0.91	0.97	0.99

Appendix 1b cont.

Power to detect increased pollution averaged across farm sites
(Testing for evidence of an average increase over time
using within control site variance as the yardstick of non-polluting variation)

Number of points per site	Number of samples per point	se mean	Size of cut-off point	0.301 (log 2)	0.477 (log 3)	0.6021 (log 4)	0.699 (log 5)
1	1	0.1056882	0.336	0.38	0.86	0.96	0.98
1	2	0.0905193	0.288	0.55	0.94	0.98	0.99
1	3	0.0848626	0.27	0.63	0.95	0.99	0.99
1	4	0.0818879	0.261	0.67	0.96	0.99	0.99
2	1	0.0776611	0.247	0.73	0.97	0.99	0.99
2	2	0.0674027	0.215	0.86	0.98	0.99	1.00
2	3	0.0636167	0.202	0.89	0.99	1.00	1.00
2	4	0.0616365	0.196	0.91	0.99	1.00	1.00
3	1	0.065714	0.209	0.87	0.99	1.00	1.00
3	2	0.0576737	0.184	0.93	0.99	1.00	1.00
3	3	0.0547317	0.174	0.95	0.99	1.00	1.00
3	4	0.0531997	0.169	0.96	0.99	1.00	1.00
4	1	0.0588377	0.187	0.93	0.99	1.00	1.00
4	2	0.0521326	0.166	0.96	1.00	1.00	1.00
4	3	0.049697	0.158	0.97	1.00	1.00	1.00
4	4	0.0484333	0.154	0.97	1.00	1.00	1.00

Appendix 1b cont.

Power to detect increased pollution at a farm site compared with control sites
(Comparing increase at one farm site with mean increase of control sites
using within control site variance as the yardstick of non-polluting variation)

Number of points per site	Number of samples per point	se mean	Size of cut-off point	0.301 (log 2)	0.477 (log 3)	0.6021 (log 4)	0.699 (log 5)
1	1	0.236326	0.752	0.08	0.16	0.29	0.42
1	2	0.2024074	0.644	0.09	0.23	0.42	0.60
1	3	0.1897586	0.604	0.10	0.28	0.50	0.67
1	4	0.1831069	0.583	0.11	0.30	0.54	0.71
2	1	0.1736555	0.553	0.12	0.35	0.60	0.77
2	2	0.150717	0.48	0.16	0.49	0.76	0.88
2	3	0.1422512	0.453	0.18	0.56	0.81	0.91
2	4	0.1378235	0.439	0.20	0.60	0.84	0.92
3	1	0.146941	0.468	0.17	0.52	0.79	0.89
3	2	0.1289622	0.41	0.23	0.68	0.88	0.94
3	3	0.1223837	0.389	0.26	0.74	0.91	0.96
3	4	0.1189582	0.379	0.28	0.77	0.92	0.96
4	1	0.1315651	0.419	0.22	0.66	0.87	0.94
4	2	0.1165721	0.371	0.30	0.78	0.93	0.97
4	3	0.1111259	0.354	0.33	0.83	0.94	0.97
4	4	0.1083001	0.345	0.36	0.85	0.95	0.98

Appendix 1b cont.

Power to detect increased pollution at farm sites compared with control sites
(Comparing mean of farm sites with mean of control sites
using within control site variance as the yardstick of non-polluting variation)

Number of points per site	Number of samples per point	se mean	Size of cut-off point	0.301 (log 2)	0.477 (log 3)	0.6021 (log 4)	0.699 (log 5)
1	1	0.1494657	0.476	0.10	0.50	0.84	0.94
1	2	0.1280137	0.407	0.16	0.75	0.94	0.98
1	3	0.1200139	0.382	0.21	0.83	0.96	0.98
1	4	0.115807	0.369	0.23	0.86	0.97	0.99
2	1	0.1098294	0.35	0.29	0.90	0.98	0.99
2	2	0.0953218	0.303	0.49	0.96	0.99	1.00
2	3	0.0899676	0.286	0.58	0.97	0.99	1.00
2	4	0.0871672	0.277	0.64	0.98	0.99	1.00
3	1	0.0929337	0.296	0.53	0.96	0.99	1.00
3	2	0.0815629	0.26	0.74	0.98	1.00	1.00
3	3	0.0774023	0.246	0.80	0.99	1.00	1.00
3	4	0.0752357	0.239	0.83	0.99	1.00	1.00
4	1	0.0832091	0.265	0.71	0.98	0.99	1.00
4	2	0.0737267	0.235	0.85	0.99	1.00	1.00
4	3	0.0702822	0.224	0.89	0.99	1.00	1.00
4	4	0.068495	0.218	0.91	0.99	1.00	1.00

APPENDIX 2: Total number of samples required for different combinations of number of points per site, samples per point and number of control sites for four farm sites.

Total number of samples required

Number of points per site	Number of samples per point	Number of control sites 2	3	4	5	6
1	1	6	7	8	9	10
1	2	12	14	16	18	20
1	3	18	21	24	27	30
1	4	24	28	32	36	40
2	1	12	14	16	18	20
2	2	24	28	32	36	40
2	3	36	42	48	54	60
2	4	48	56	64	72	80
3	1	18	21	24	27	30
3	2	36	42	48	54	60
3	3	54	63	72	81	90
3	4	72	84	96	108	120
4	1	24	28	32	36	40
4	2	48	56	64	72	80
4	3	72	84	96	108	120
4	4	96	112	128	144	160